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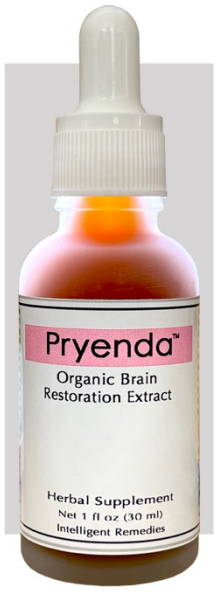
Product Information Sheet



Pryenda™ is a Phytotherapeutic extraction of***, Sculetaria baichenalis, Gastrodia elata, Illicium venum***, ***Vitis vinifera***, ***Camellia sinensis, Paonia alba and Cinnamomum verum***. Using advanced laboratory extraction apparatus & proprietary production protocols, these phytochemicals are known to stop the formation of prions in the brain.

Transmissible spongiform encephalopathies (TSE) are characterized by the misfolding of the host encoded prion protein (PrP(C)) into a pathogenic isoform (PrP(Sc)) which leads to the accumulation of β-sheet-rich fibrils and subsequent loss of neurons and synaptic functions. The traditional medicinal herb Scutellaria *baichenalis* (S. *baichenalis)* has natural compounds, the flavonoids baicalein and baicalin, that effect the development of prion disease. S. *baichenalis* extracts as well as the individual compounds are therapeutic treatments against TSEs and other neurodegenerative diseases like Alzheimer's and Parkinson's disease.

As the occurrence of dementia and cardiovascular disease increases the development of Alzheimer's disease (AD), vascular dementia (VD), and other cardiovascular diseases (CD) also increases. The number of patients suffering from AD, VD, and CD is a significant threat to the aging people all over the world. The use of medicinal herbs has a long history in Asia and is commonly used to treat various neurological diseases including stroke, epilepsy and VD. Listed in the earliest known Chinese Materia, the Bencao gangmu (Compendium of Materia Medica), the most renowned herbal text in China, is Gastrodia elata Blume (Tianma).

Progressive accumulation of misfolded amyloid proteins in intracellular and extracellular spaces is one of the principal reasons for synaptic damage and impairment of neuronal communication in several neurodegenerative diseases. The natural polyphenols found in *Vitis vinifera* have been shown to be anti-amyloid, anti-inflammatory and neuroprotective agent for several neurodegenerative diseases. Because of its pleotropic actions on the central nervous system, including preferential binding to amyloid proteins, It is a treatment for age-related brain diseases. Cur reduces amyloid burden, rescues neuronal damage, and restores normal cognitive and sensory motor functions in different animal models of neurodegenerative diseases inckuding Alzheimer's, Parkinson's, Huntington's, and prion diseases.

Pryenda™ is uniquely extracted from organic herbs using organic cane alcohol and deep ocean mineral water, as the extraction solvent. Utilizing advanced all-glass apparatus **Pryenda™** undergoes hours of reflux extraction that applies heat and organic ethanol to enhance the bioavailability of the resultant extraction.

Pryenda™ Contains:

Sculetaria baichenalis, Radix Scutellariae is the dried root of the medicinal plant Scutellariae baicalensis It exhibits a variety of therapeutic effects and has a long history of application in traditional formulations as well as in modern herbal medications. It has been confirmed that flavonoids are the most abundant

constituents and induce these therapeutic effects. Six flavones are proven to be the major bioactive flavones in Radix Scutellariae existing in the forms of aglycones (baicalein, wogonin, oroxylin A) and glycosides (baicalin, wogonoside, oroxylin A-7-glucuronide). All six flavones are pharmacologically active and show great potential in the treatment of inflammation, cancers and virus-related diseases.

Gastrodia elata (Tianma) is a traditional Chinese medicine often used for the treatment of headache, convulsions, hypertension, and cardiovascular diseases. The vasodilatory actions of Tianma has specific effects on memory and learning as well as on Alzheimer's disease (AD)-related signaling. Tianma enhances cognitive functions and the α-secretase-mediated proteolytic processing of the amyloid precursor protein (App) that precludes the amyloid-β peptide production and supports the non-amyloidogenic processing of App which is favorable in AD treatment. Tianma promotes cognitive functions and neuronal survival by inhibiting β-site App-cleaving enzyme 1 activity and promoting the neuroprotective α-secretase activity.

Illicium venum, Star Anise, contains Shikimic acid, which exhibits a dose-dependent inhibitory effect on platelet aggregation. Shikimic acid derivatives have also been shown to exhibit useful biological activity. Most notably, the well-known antiviral drug oseltamivir (Tamiflu), which acts as a viral neuraminidase inhibitor, is used to treat seasonal influenza and has been deployed during H1N1 influenza outbreaks. It displays anticancer, antiviral and antibiotic behavior, and also exhibits anticoagulant and antithrombotic activity.

Vitis vinifera, (grape) contains various phenolic compounds, flavonoids and stilbenes. The active constituents of different parts of V. vinifera and their pharmacological effects include skin protection, antioxidant, antibacterial, anticancer, anti-inflammatory and antidiabetic activities, as well as hepatoprotective, cardioprotective and neuroprotective effects. Grape seeds are a valuable source of phenolic compounds including resveratrol (RSV). Grape and one of its biologically active constituents, RSV, exert their protective effects against different natural or chemical toxins which could alter physiological homeostasis through a variety of mechanisms. Some of these mechanisms of actions include increase in superoxide dismutase, hemeoxygenase-1, and glutathione peroxidase activities and reduced glutathione content and decrease in malondialdehyde (MDA) levels and activation of the nuclear erythroid2-related factor2/ARE pathway. There are also various reports of the potential use of such compounds in preventing different ailments including cardiovascular diseases, cancer, degenerative diseases, and inflammatory disorders.

Camellia sinensis a major component of green tea, EGCG is famous for its anti-inflammatory and anti-apoptotic properties. EGCG, as a potent inducer of HO-1, can suppress renal injury by reducing oxidative stress and inflammation.

Paeoniae alba, also known as Chinese peony, is a TCM plant used to treat inflammatory disorders, and has been proven to be effective in the treatment of rheumatoid arthritis (RA), systemic lupus erythematosus, hepatitis, and other inflammatory/autoimmune diseases. It is effective in relieving pain in RA models. Multiple pathways may be involved in the analgesic effect of ***Paeoniae***, one of them being its anticholinergic action. The effect might also be mediated by adenosine A1 receptors, κ-opioid receptors, and α2-adrenoceptors.

Cinnamomum verum Contains antioxidants, including polyphenols, phenolic acid and flavonoids. These compounds work to fight oxidative stress in the body and aid in the prevention of chronic disease.

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Worse Than the Disease? Reviewing Some Possible Unintended Consequences of the mRNA Vaccines Against COVID-19

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ABSTRACT

Operation Warp Speed brought to market in the United States two mRNA vaccines, produced by Pfizer and Moderna. Interim data suggested high efficacy for both of these vaccines, which helped legitimize Emergency Use Authorization (EUA) by the FDA. However, the exceptionally rapid movement of these vaccines through controlled trials and into mass deployment raises multiple safety concerns. In this review we first describe the technology underlying these vaccines in detail. We then review both components of and the intended biological response to these vaccines, including production of the spike protein itself, and their potential relationship to a wide range of both acute and long-term induced pathologies, such as blood disorders, neurodegenerative diseases and autoimmune diseases. Among these potential induced pathologies, we discuss the relevance of prion-protein-related amino acid sequences within the spike protein. We also present a brief review of studies supporting the potential for spike protein "shedding", transmission of the protein from a vaccinated to an unvaccinated person, resulting in symptoms induced in the latter. We finish by addressing a common point of debate, namely, whether or not these vaccines could modify the DNA of those receiving the vaccination. While there are no studies demonstrating definitively that this is happening, we provide a plausible scenario, supported by previously established pathways for transformation and transport of genetic material, whereby injected mRNA could ultimately be incorporated into germ cell DNA for transgenerational transmission. We conclude with our recommendations regarding surveillance that will help to clarify the long-term effects of these experimental drugs and allow us to better assess the true risk/benefit ratio of these novel technologies.

Keywords: *antibody dependent enhancement, autoimmune diseases, gene editing, lipid nanoparticles, messenger RNA, prion diseases, reverse transcription, SARS-CoV-2 vaccines*

Introduction

Unprecedented. This word has defined so much about 2020 and the pandemic related to SARS-CoV-2. In addition to an unprecedented disease and its global response, COVID-19 also initiated an unprecedented process of vaccine research, production, testing, and public distribution (Shaw,

ARTICLES
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nature neuroscience

The S1 protein of SARS-CoV-2 crosses the blood–brain barrier in mice

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It is unclear whether severe acute respiratory syndrome coronavirus 2, which causes coronavirus disease 2019, can enter the brain. Severe acute respiratory syndrome coronavirus 2 binds to cells via the S1 subunit of its spike protein. We show that intravenously injected radioiodinated S1 (I-S1) readily crossed the blood–brain barrier in male mice, was taken up by brain regions and entered the parenchymal brain space. I-S1 was also taken up by the lung, spleen, kidney and liver. Intranasally administered I-S1 also entered the brain, although at levels roughly ten times lower than after intravenous administration. *APOE* genotype and sex did not affect whole-brain I-S1 uptake but had variable effects on uptake by the olfactory bulb, liver, spleen and kidney. I-S1 uptake in the hippocampus and olfactory bulb was reduced by lipopolysaccharide-induced inflammation. Mechanistic studies indicated that I-S1 crosses the blood–brain barrier by adsorptive transcytosis and that murine angiotensin-converting enzyme 2 is involved in brain and lung uptake, but not in kidney, liver or spleen uptake.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease 2019 (COVID-19) pandemic. In addition to pneumonia and acute respiratory distress, COVID-19 is associated with a host of symptoms that relate to the CNS, including loss of taste and smell, headaches, twitching, seizures, confusion, vision impairment, nerve pain, dizziness, impaired consciousness, nausea and vomiting, hemiplegia, ataxia, stroke and cerebral hemorrhage[1,2]. It has been postulated that some of the symptoms of COVID-19 may be due to direct actions of the virus on the CNS; for example, respiratory symptoms could be in part due to SARS-CoV-2 invading the respiratory centers of the brain[1,3]. Encephalitis has also been reported in COVID-19, and could be a result of virus or viral proteins having entered the brain[4,5]. SARS-CoV-2 mRNA has been recovered from the cerebrospinal fluid[4], suggesting it can cross the blood–brain barrier (BBB). Other coronaviruses, including the closely related SARS virus that caused the 2003–2004 outbreak, are able to cross the BBB[6–8], and SARS-CoV-2 can infect neurons in a BrainSphere model[9]. However, SARS-CoV-2 could induce changes in the CNS without directly crossing the BBB, as COVID-19 is associated with a cytokine storm, and many cytokines cross the BBB to affect CNS function[10].

Here we assess whether one viral protein of SARS-CoV-2, the spike 1 protein (S1), can cross the BBB. This question is important and clinically relevant for two reasons. First, some proteins shed from viruses have been shown to cross the BBB, inducing neuroinflammation and otherwise impairing CNS functions[11–17]. Second, the viral protein that binds to cells can be used to model the activity of the virus; in other words, if the viral binding protein crosses the BBB, it is likely that protein enables the virus to cross the BBB as well[18,19]. S1 is the binding protein for SARS-CoV-2 (ref. [20]); it binds to angiotensin-converting enzyme 2 (ACE2)[21–23] and probably other proteins as well.

In this study, we show that I-S1 readily crossed the murine BBB, entered the parenchymal tissue of the brain and, to a lesser degree, was sequestered by brain endothelial cells and associated with the brain capillary glycocalyx. We describe I-S1 rate of entry into the brain after intravenous (i.v.) and intranasal administration, determine its uptake in 11 different brain regions, examine the effect of inflammation, *APOE* genotype and sex on I-S1 transport, and compare I-S1 uptake in the brain to the uptake in the liver, kidney, spleen and lung. Based on experiments with the glycoprotein WGA, we found that brain entry of I-S1 likely involves the vesicular-dependent mechanism of adsorptive transcytosis.

Results

I-S1 protein is transported across the mouse blood–brain barrier. We obtained S1 proteins from two commercial sources: RayBiotech and AMSBIO. The S1 proteins were radiolabeled in-house, and verified to be intact after labeling by autoradiography gels (Extended Data Fig. 1 and Supplementary Fig. 1). We determined whether intravenously injected I-S1 could cross the BBB in mice, by measuring its blood-to-brain influx constant (K_i) using multiple-time regression analysis (MTRA). MTRA plots the tissue/serum ratios for I-S1 against exposure time, which is a measure of time that has been corrected for the clearance of I-S1 from blood. The slope of the linear portion of this plot measures K_i, that is, the unidirectional influx constant for I-S1.

We co-injected ^{99m}Tc-labeled albumin (T-Alb) along with the I-S1. T-Alb crosses the intact BBB poorly and so can be used to measure the vascular space of the brain. Any brain/serum ratios for I-S1 that exceed the brain/serum ratios of T-Alb therefore represent extravascular I-S1; that is, material which has crossed the BBB. T-Alb can also be used to measure the leakiness of peripheral tissue beds and of a BBB that has been disrupted by disease or inflammation.

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A systems approach to prion disease

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Prions cause transmissible neurodegenerative diseases and replicate by conformational conversion of normal benign forms of prion protein (PrP^{C}) to disease-causing PrP^{Sc} isoforms. A systems approach to disease postulates that disease arises from perturbation of biological networks in the relevant organ. We tracked global gene expression in the brains of eight distinct mouse strain–prion strain combinations throughout the progression of the disease to capture the effects of prion strain, host genetics, and PrP concentration on disease incubation time. Subtractive analyses exploiting various aspects of prion biology and infection identified a core of 333 differentially expressed genes (DEGs) that appeared central to prion disease. DEGs were mapped into functional pathways and networks reflecting defined neuropathological events and PrP^{Sc} replication and accumulation, enabling the identification of novel modules and modules that may be involved in genetic effects on incubation time and in prion strain specificity. Our systems analysis provides a comprehensive basis for developing models for prion replication and disease, and suggests some possible therapeutic approaches.
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Subject Categories: neuroscience; molecular biology of disease
Keywords: microarray; network analysis; neurodegenerative disease; prion

Introduction

Systems approaches to disease arise from a simple hypothesis—disease emerges from the functioning of one or more disease-perturbed networks (genetic and/or environmental perturbations) that alter the levels of proteins and other informational molecules controlled by these networks. The dynamically changing levels of disease-perturbed proteins (networks) across disease progression presumably explain the mechanisms of the disease. Systems approaches to biology or medicine have two cardinal features: (1) global analyses to generate comprehensive data sets in the disease-relevant organ or cells across the dynamically changing disease process (e.g. all mRNA, miRNA, or protein levels) and (2) the integration of different levels of biological information (DNA, mRNA, miRNA, protein, interactions, metabolites, networks, tissues, organs, and phenotypes) to generate hypotheses about the fundamental principles of the disease (Hood *et al*, 2004). In this study, we present a systems biology approach that effectively uses these two features, uses multiple inbred mouse strains, uses a deep understanding of prion biology and applies statistical data integration methods to deal with two striking challenges: (1) sorting out the signal-to-noise issues arising from the global disease-associated changes as both measurement noise and biological noise and (2) reducing enormous data dimensionality so that the processes can be identified and visualized for study. The keys to reducing this noise are to apply a deep understanding of biology to carry out subtractive data analyses that focus on particular biological issues—as well as to use integrative statistical methods. We applied the systems approach to experimentally tractable neurodegenerative diseases caused by prion infection of mice. Analysis of a large data set (~20 million data points) revealed slightly more than 300 differentially expressed genes (DEGs) that may encode fundamental processes in prion disease.

Prions are unique among transmissible, disease-causing agents in that they replicate by conformational conversion of normal benign forms of prion protein (PrP^{C}) to disease-specific

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A medicinal herb *Scutellaria lateriflora* inhibits PrP replication *in vitro* and delays the onset of prion disease in mice

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Transmissible spongiform encephalopathies (TSE) are characterized by the misfolding of the host encoded prion protein (PrP^{C}) into a pathogenic isoform (PrP^{Sc}) which leads to the accumulation of β-sheet-rich fibrils and subsequent loss of neurons and synaptic functions. Although many compounds have been identified which inhibit accumulation or dissolve fibrils and aggregates *in vitro* there is no therapeutic treatment to stop these progressive neurodegenerative diseases. Here we describe the effects of the traditional medicinal herb *Scutellaria lateriflora* (*S. lateriflora*) and its natural compounds, the flavonoids baicalein and baicalin, on the development of prion disease using *in vitro* and *in vivo* models. *S. lateriflora* extract as well as both constituents reduced the PrP^{res} accumulation in scrapie-infected cell cultures and cell-free conversion assays and lead to the destabilization of pre-existing PrP^{Sc} fibrils. Moreover, tea prepared from *S. lateriflora*, prolonged significantly the incubation time of scrapie-infected mice upon oral treatment. Therefore *S. lateriflora* extracts as well as the individual compounds can be considered as promising candidates for the development of new therapeutic drugs against TSEs and other neurodegenerative diseases like Alzheimer's and Parkinson's disease.

Keywords: prion protein, inhibitor, *Scutellaria lateriflora*, baicalein, baicalin

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases which are characterized by the accumulation and deposition of a pathogenic isoform (PrP^{Sc}) of the host encoded cellular prion protein (PrP^{C}) designated PrP^{Sc}. Both isoforms share the same amino acid sequence but differ in conformation, resistance to proteinase K (PK), and pathogenicity. PrP^{Sc} tends to oligomerize by a seeded polymerization mechanism followed by the formation of multimers and eventually of fibril structures. This aggregation can be reproduced in an analogous manner *in vitro*, using cell-based as well as cell-free assays. The evolved PrP^{Sc} like isoforms – termed PrP^{res} – harbor similar biochemical characteristics like resistance to Proteinase K and detection by same antibodies.

Accumulation of PrP^{Sc} in the central nervous system (CNS) is accompanied by neurological dysfunctions, neuronal vacuolation, and astrocytic gliosis. Although the exact disease causing mechanism is unknown to date, there is evidence for a general neurotoxicity of these aggregates, which deteriorate synaptic function and induce oxidative stress and membrane disruption (Soto and Estrada, 2008). Prion diseases belong to the group of protein misfolding diseases like Alzheimer's (AD), Parkinson's (PD), and Huntington's disease (HD), which are generally characterized by an incorrect folding process of a host encoded protein with a conformation different from its native structure. The misfolding is followed by a self-aggregation and polymerization of the protein according to a "seeding-nucleation" process (Jarrett and Lansbury, 1993).

As PrP^{Sc} formation and aggregation is the central event in prion diseases, the inhibition of oligomer formation and fibril extension as well as the enhancement of fibril degradation are major targets for the development of therapeutic strategies against TSEs. Several substances have been identified which inhibit PrP^{res} formation and accumulation *in vitro* and prolonged survival in scrapie-infected animals: Congo red (Caughey and Race, 1992), branched polyamines (Supattapone et al., 2001), porphyrins and phthalocyanines (Priola et al., 2000; Caughey et al., 2007), heparan sulfate mimetics (Adjou et al., 2003), amphotericin (Mange et al., 2000), curcumine (Caughey et al., 2003; Yang et al., 2009), and tetracyclines (De Luigi et al., 2008). However, for various reasons none of these compounds has been included in prevention and treatment regimes for humans yet. Most recently two new substance classes, benzothiazoles (Geissen et al., 2011) and diphenylpyrazoles (Leidel et al., 2011), were identified by high-throughput screening approaches that inhibit PrP^{res} accumulation in cell culture models and prolong incubation times in scrapie-infected mice.

Other therapeutic strategies rely on passive immunization (White et al., 2003), RNA interference (Pfeifer et al., 2006), RNA aptamers (Proske et al., 2002), copper chelating antibiotics (Murakami-Kubo et al., 2004), or on the induction of autophagy by Lithium (Heiseke et al., 2009).

Recent studies on AD suggest that phenolic compounds like green tea epigallocatechin gallate (Rezai-Zadeh et al., 2005), herb extracts like grape seed polyphenolic extract (Wang et al., 2009; Liu et al., 2011), or medicinal herbs like *Paeonia suffruticosa* (Fujiwara

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Original Article

Gastrodia elata Blume (tianma) mobilizes neuro-protective capacities

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Abstract: Tianma (*Gastrodia elata Blume*) is a traditional Chinese medicine (TCM) often used for the treatment of headache, convulsions, hypertension and neurodegenerative diseases. Tianma also modulates the cleavage of the amyloid precursor protein App and cognitive functions in mice. The neuronal actions of tianma thus led us to investigate its specific effects on neuronal signalling. Accordingly, this pilot study was designed to examine the effects of tianma on the proteome metabolism in differentiated mouse neuronal N2a cells using an iTRAQ (isobaric tags for relative and absolute quantitation)-based proteomics research approach. We identified 2178 proteins, out of which 74 were found to be altered upon tianma treatment in differentiated mouse neuronal N2a cells. Based on the observed data obtained, we hypothesize that tianma could promote neuro-regenerative processes by inhibiting stress-related proteins and mobilizing neuroprotective genes such as Nxn, Dbnl, Mobkl3, Clic4, Mki67 and Bax with various regenerative modalities and capacities related to neuro-synaptic plasticity.

Keywords: Aging, tianma, neuron, neurodegeneration, metabolism, signalling, TCM

Introduction

Since recent data show that the number of people affected by Alzheimer's disease (AD) and dementia is increasing at an epidemic pace, there has been a interest in developing novel protective agents because biological aging also represents the major risk factor with respect to the development of AD, vascular dementia (VD) and other cardiovascular diseases (CD). Traditional herbal medicine is especially attractive for disease prevention, health maintenance, and sicknesses that are non-responsive to current Western medicine and thus has potential benefits that attract worldwide attention and interests. The use of medicinal herbs has a long history in Asia and is commonly used to treat various neurological diseases including stroke, epilepsy and VD [1-3]. Orchids and their derivatives have been shown to benefit the improvement of neural functions in clinical studies but the underlying mechanisms are largely unknown which severely hampered the more extensive application of such potential drugs as well as the potential of industrial exploitation of it [4-6]. According to ancient Chinese medical literature, tianma (*Gastrodia elata Blume,* Orchidaceae) is a herbal medicine for the control of the internal movement of wind. The dry tuber of tianma has long been officially listed in the Chinese Pharmacopoeia and is used in treating headaches, dizziness, tetanus, epilepsy, infantile convulsions and numbness of the limbs [4, 6-11]. Previously, we could demonstrate *in vivo* the potential neuro-protective action of tianma and its capacity to enhance cognitive functions in mice [12].

Recently, we have successfully applied the two dimensional (2D) liquid chromatography coupled with tandem mass spectrometry-based isobaric tag for relative and absolute quantification (2D-LC-MS/MS-iTRAQ) strategy in the area of neuro-degenerative diseases [13, 14]. Our group has recently reported the facilitating effect of tianma on α-secretase-mediated cleav-

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Original Article

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Gastrodia elata modulates amyloid precursor protein cleavage and cognitive functions in mice

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Summary ***Gastrodia elata*** **(Tianma) is a traditional Chinese medicine often used for the treatment of headache, convulsions, hypertension, and cardiovascular diseases. The vasodilatory actions of Tianma led us to investigate its specific effects on memory and learning as well as on Alzheimer's disease (AD)-related signaling. We conducted a radial arm water maze analysis and the novel object recognition test to assess the cognitive functions of Tianma-treated mice. Our data show that Tianma enhances cognitive functions in mice. Further investigations revealed that Tianma enhances the α-secretase-mediated proteolytic processing of the amyloid precursor protein (App) that precludes the amyloid-β peptide production and supports the non-amyloidogenic processing of App which is favorable in AD treatment. We hypothesize that Tianma promotes cognitive functions and neuronal survival by inhibiting β-site App-cleaving enzyme 1 activity and promoting the neuroprotective α-secretase activity.**

Keywords: Alzheimer's disease, β-Amyloid precursor protein, Kampo, Neurodegeneration, traditional Chinese medicine

1. Introduction

As the occurrence of dementia and cardiovascular disease increase with age, there has been a growing interest in developing novel protective agents because biological aging represents also the major risk factor with respect to the development of Alzheimer's disease (AD), vascular dementia (VD), and other cardiovascular diseases (CD). The number of patients suffering from AD, VD, and CD is a significant threat to the aging people all over the world. However, despite advances in technology and understanding of biological systems, drug discovery for these and other diseases is still a lengthy and expensive process. Traditional herbal medicine is especially attractive for disease prevention, health maintenance, and sicknesses that are non-responsive to current Western medicine and thus has potential benefits that attract worldwide attention and interests. The use of medicinal herbs has a long history in Asia and is commonly used to treat various neurological diseases including stroke, epilepsy and VD (*1,2*). A total of 365 plants including several orchids are listed in the earliest known Chinese Materia Medica (Shennon bencaojing (~ 100AD) or Divine Husbandman's Classic of the Materia Medica). In Bencao gangmu (Compendium of Materia Medica), the most renowned herbal text in China, three orchids that have been extensively studied and widely used as herbal medicines are *Dendrobium nobile* (Shi Hu/Shifu), *Gastrodia elata Blume* (Tianma, Orchidaceae), and *Bletilla striata*. However, current Western methodologies need to take into consideration the complex mixture of chemicals and how they are to be used in human. The scientific proof and clinical validation of these herbal formulations require a rigorous approach that includes chemical standardization, biological assays, animal models, and clinical trials (*3,4*).

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Review

Use of Curcumin, a Natural Polyphenol for Targeting Molecular Pathways in Treating Age-Related Neurodegenerative Diseases

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Abstract: Progressive accumulation of misfolded amyloid proteins in intracellular and extracellular spaces is one of the principal reasons for synaptic damage and impairment of neuronal communication in several neurodegenerative diseases. Effective treatments for these diseases are still lacking but remain the focus of much active investigation. Despite testing several synthesized compounds, small molecules, and drugs over the past few decades, very few of them can inhibit aggregation of amyloid proteins and lessen their neurotoxic effects. Recently, the natural polyphenol curcumin (Cur) has been shown to be a promising anti-amyloid, anti-inflammatory and neuroprotective agent for several neurodegenerative diseases. Because of its pleotropic actions on the central nervous system, including preferential binding to amyloid proteins, Cur is being touted as a promising treatment for age-related brain diseases. Here, we focus on molecular targeting of Cur to reduce amyloid burden, rescue neuronal damage, and restore normal cognitive and sensory motor functions in different animal models of neurodegenerative diseases. We specifically highlight Cur as a potential treatment for Alzheimer's, Parkinson's, Huntington's, and prion diseases. In addition, we discuss the major issues and limitations of using Cur for treating these diseases, along with ways of circumventing those shortcomings. Finally, we provide specific recommendations for optimal dosing with Cur for treating neurological diseases.

Keywords: neurodegenerative diseases; amyloidosis; curcumin; neuroinflammation; anti-amyloid; molecular chaperones; natural polyphenol

1. Introduction

Aggregation of misfolded amyloid proteins and their deposition in intracellular and extracellular spaces of the central nervous system (CNS) are associated with several neurological diseases, including Alzheimer's (AD), Parkinson's (PD), Huntington's (HD) and prion diseases [1,2]. Most of these diseases are age-related, complicated disorders which involve a multitude of causative factors, including neuroinflammation [3], oxidative damage and deposition of misfolded protein aggregates [4]. These events can occur separately or together or in causing neuronal degeneration, which leads to perturbation of neuronal communications, resulting in long-term cognitive and motor dysfunction. The neuropathological onset of these diseases may have occurred long before the manifestation of

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Inhibition of Protease-Resistant Prion Protein Accumulation In Vitro by Curcumin

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Inhibition of the accumulation of protease-resistant prion protein (PrP-res) is a prime strategy in the development of potential transmissible spongiform encephalopathy (TSE) therapeutics. Here we show that curcumin (diferoylmethane), a major component of the spice turmeric, potently inhibits PrP-res accumulation in scrapie agent-infected neuroblastoma cells (50% inhibitory concentration, ~10 nM) and partially inhibits the cell-free conversion of PrP to PrP-res. In vivo studies showed that dietary administration of curcumin had no significant effect on the onset of scrapie in hamsters. Nonetheless, other studies have shown that curcumin is nontoxic and can penetrate the brain, properties that give curcumin advantages over inhibitors previously identified as potential prophylactic and/or therapeutic anti-TSE compounds.

Transmissible spongiform encephalopathies (TSE) or prion diseases are untreatable, fatal neurodegenerative diseases that include bovine spongiform encephalopathy, chronic wasting disease, scrapie, and Creutzfeldt-Jakob disease. A central event in TSE diseases is the conversion of the normal, protease-sensitive isoform of prion protein (PrP-sen or PrP^{C}) to an abnormal, protease-resistant form, PrP-res or PrP^{Sc}. Numerous compounds have been identified as inhibitors of PrP-res formation in scrapie agent-infected murine neuroblastoma (ScNB) cells (1–3, 5). The most potent of these inhibitors can also protect rodents against scrapie if they are administered near the time of infection (7, 8, 10, 11, 14). Unfortunately, none of these compounds are known to be both safe and effective for use in humans and animals (8, 10, 11). One therapeutic weakness of most of these compounds is likely an inability to penetrate the brain where most of the PrP-res accumulates and TSE pathogenesis occurs.

Curcumin, the major component of the spice turmeric and the yellow pigment in curry powder, has several properties that make it of interest as a possible anti-TSE drug. First, its structure resembles Congo red, the most potent of the small-molecule PrP-res inhibitors that have been assayed in ScNB cells (Fig. 1) in that both are potentially planar compounds that have two aromatic rings or ring systems with conjugated linkers. Structure-activity studies have provided evidence that the potential for coplanarity of the rings and linker is important for the inhibitory potency of Congo red (6). Second, unlike Congo red, curcumin is uncharged and is thought to have at least limited bioavailability to the brain after consumption. Indeed, recent studies with a rat model of Alzheimer's disease reported that dietary curcumin reduces β-peptide deposition in the brain as well as associated neuropathology and cognitive deficits (9, 12). Third, curcumin has antioxidant activity, a factor that may be important given that oxidative damage is a feature in TSE neuropathogenesis (13). Fourth, humans consume curcumin in large amounts with no apparent toxicity. Toxicology studies have indicated that rodents can tolerate for a long period up to 5% of their diet being turmeric oleoresin (~80% curcumin) without their life spans being shortened (http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr427.html). These considerations prompted us to test whether curcumin could inhibit the formation and accumulation of PrP-res.

Curcumin was added to the culture medium of ScNB cells seeded at a density of 1/10 confluence and grown to near confluence for 3 to 4 days. Approximately 90% of the ScNB cells used to seed these cultures were infected, as was indicated by the fact 9 out of 10 single-cell subclones from concurrent passes of this ScNB cell line were highly positive for PrP-res (data not shown). The accumulation of PrP-res during the growth of the ScNB cultures was assayed by detergent extraction, proteinase K (PK) treatment, and immunoblotting by previously described methods (5). A curcumin concentration-dependent reduction of PrP-res accumulation was observed with a concentration giving half maximal inhibition of ~10 nM (Fig. 2A and B). This 50% inhibitory concentration rivals that of Congo red (~10 nM) (2, 3) and is 2,500-fold lower than the concentration of curcumin (25 μM) that began to show evidence of cytotoxicity in the ScNB cells (not shown). The curcumin-induced reduction in PrP-res was long-lasting because the PrP-res levels in cultures treated with 1 μM curcumin for 4 days (one pass) remained low after four subsequent passes in the absence of curcumin (Fig. 2C). The observed effects of curcumin were not due to artifactual interference with the detection of PrP-res or an enhancement of the protease sensitivity of PrP-res after lysis of the cells, because addition of 1 μM curcumin to the otherwise untreated control cell lysates prior to PK digestion did not affect the amount of PrP-res detected (Fig. 2D). Furthermore, incubation of purified hamster 263K strain PrP-res (0.13 μM) with curcumin concentrations of up to 5 mM in 50 mM Tris-HCl–150 mM NaCl, pH 8, for 16 h at room temperature did not enhance its sensitivity to PK or reduce its detection by immunoblotting (data not

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Natural product-based amyloid inhibitors

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Abstract

Many chronic human diseases, including multiple neurodegenerative diseases, are associated with deleterious protein aggregates, also called protein amyloids. One common therapeutic strategy is to develop protein aggregation inhibitors that can slow down, prevent, or remodel toxic amyloids. Natural products are a major class of amyloid inhibitors, and several dozens of natural product-based amyloid inhibitors have been identified and characterized in recent years. These plant- or microorganism-extracted compounds have shown significant therapeutic potential from *in vitro* studies as well as *in vivo* animal tests. Despite the technical challenges of intrinsic disordered or partially unfolded amyloid proteins that are less amenable to characterizations by structural biology, a significant amount of research has been performed, yielding biochemical and pharmacological insights into how inhibitors function. This review aims to summarize recent progress in natural product-based amyloid inhibitors and to analyze their mechanisms of inhibition *in vitro*. Major classes of natural product inhibitors and how they were identified are described. Our analyses comprehensively address the molecular interactions between the inhibitors and relevant amyloidogenic proteins. These interactions are delineated at molecular and atomic levels, which include covalent, non-covalent, and metal-mediated mechanisms. *In vivo* animal studies and clinical trials have been summarized as an extension. To enhance natural product bioavailability *in vivo*, emerging work using nanocarriers for delivery has also been described. Finally, issues and challenges as well as future development of such inhibitors are envisioned.

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Disclosure

The authors report no financial conflicts of interest in this work.