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Neuroprotective effects of geniposide on Alzheimer's disease pathology

Abstract: A growing body of evidence has linked two of the most common aged-related diseases: type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD). It has led to the notion that drugs developed for the treatment of T2DM may be beneficial in modifying the pathophysiology of AD. As a receptor agonist of glucagon-like peptide-1 (GLP-1R), which is a newer drug class to treat T2DM, geniposide shows clear effects in inhibiting pathological processes underlying AD, such as promoting neurite outgrowth. In the present article, we review the possible molecular mechanisms of geniposide to protect the brain from pathologic damages underlying AD: reducing amyloid plaques, inhibiting τ phosphorylation, preventing memory impairment and loss of synapses, reducing oxidative stress and the chronic inflammatory response, and promoting neurite outgrowth via the GLP-1R signaling pathway. In summary, the Chinese herb geniposide shows great promise as a novel treatment for AD.

Keywords: Alzheimer's disease; amyloid-β; geniposide; glucagon-like peptide receptor; neurofibrillary tangles; neuroprotection; oxidative stress; τ protein; type 2 diabetes mellitus.

DOI 10.1515/revneuro-2015-0005
Received February 1, 2015; accepted February 25, 2015; previously published online April 16, 2015

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Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of progressive cognitive decline in the aged population. The characteristic pathological hallmarks are the abundance of two abnormal aggregated proteins in brain tissue: neurofibrillary tangles (NFTs) composed mainly of the microtubule-associated protein τ and amyloid plaques composed of insoluble amyloid-β (Aβ) deposits, synaptic and neuronal loss, as well as dysfunction associated to the neurochemical changes in brain tissue (Mathis et al., 2007). The multiple molecular pathogenic changes contributing to the pathological hallmarks of AD include mitochondrial dysfunction, oxidative stress, endoplasmic reticulum (ER) stress, and inflammation, which lead to the varying levels of plaques and tangles, and these studies also explain the relationships between protein aggregation and neuronal loss in neurodegeneration (Stalder et al., 1999; Meares et al., 2011).

The current pharmacotherapy of AD is limited to cholinesterase inhibitors and the *N*-methyl-D-aspartate antagonist memantine. Although these drugs have been shown to treat the symptoms of AD, they have not been shown to cease or reverse the pathophysiological causes of AD (Tan et al., 2014; Werner and Altaf, 2015). The present medications approved by the Food and Drug Administration do little to slow disease progression and provided no indication for the underlying progressive loss of synaptic connections and neurons (Wright et al., 2014). Thus, it is of great importance to seek novel therapeutic agents. To find new medications to treat AD based on our molecular pathology, the knowledge of AD has become a priority in the AD area of research. Priority candidate treatments, for which there is considered to be a high level of supportive evidence, such as antihypertensives, antibiotics, antidiabetic drugs, and retinoid therapy, have been summarized and described (Corbett et al., 2012).

Considering type 2 diabetes mellitus (T2DM) had been identified as a risk factor for AD, it is possible to develop drugs that can treat T2DM to also treat AD. The use of long-lived mimetics of the glucagon-like peptide-1 (GLP-1) that are resistant to cleavage by proteases is a successful strategy to treat T2DM. In the present review, we

explore a possibility to develop a new strategy to treat AD using receptor agonists of GLP-1R and explain the possible molecular mechanism. Epidemiological studies found a correlation between an increased risk of developing AD and T2DM (Ristow, 2004; Biessels et al., 2006; Haan, 2006). Further research showed a range of shared pathophysiological changes seen in T2DM and AD (Akter et al., 2011). The possible common or interactive processes in T2DM and AD have been reviewed (Nelson and Alkon, 2005; Li and Holscher, 2007).

There are currently ongoing clinical trials that test the effectiveness of 'antidiabetic' drugs in AD patients. We are aware of two ongoing pilot studies of GLP-1 analogues for AD. A clinical trial of exendin-4 in AD is performed by the National Institute on Aging (ClinicalTrials identifier: NCT01255163). The other is evaluating liraglutide in AD conducted by the Imperial College London (ClinicalTrials identifier: NCT01843075). Three metabolic hormones have shown promise in preclinical models of AD: amylin, leptin, and GLP-1. The neuroprotective effects of GLP-1 and its analogues have shown considerable results *in vivo* and *in vitro* (Wang et al., 2010; Hölscher, 2014a). The GLP-1 analogue liraglutide showed protective effects from memory impairments in the amyloid precursor protein (APP)/presenilin 1 (PS1) mouse model of AD. The Aβ levels, plaque load, and the inflammation response in the brain were much reduced after treatment by liraglutide. Furthermore, memory formation and synaptic plasticity in the hippocampus were rescued by the drug (McClean et al., 2011). The drug also reversed some long-term damage in very old transgenic AD mice (McClean et al., 2014a). The newer GLP-1 analogue lixisenatide also had these impressive neuroprotective effects (McClean et al., 2014b). A study characterized the effects of another GLP-1R agonist, exendin-4, on stress-induced toxicity in neuronal cultures and on Aβ and τ levels in triple transgenic AD (3xTg-AD) mice with and without streptozocin (STZ)-induced diabetes (Li et al., 2010). Liraglutide, exendin-4, and lixisenatide are all on the market in Europe as treatments for diabetes. Together, these results indicated a potential effect of GLP-1R agonists in treating AD, particularly when associated with T2DM or glucose intolerance (Hölscher, 2014b).

Geniposide, a key component extracted from the fruit of *Gardenia jasminoides* Ellis, is a major iridoid glycoside considered to be responsible for various biological effects of the herbs, and its aglycon is genipin. Gardenia is a widely used Chinese herb for the treatment of hepatic disease, inflammation disorders, contusions and brain disorders (Wang et al., 1992; Chen et al., 2010; Wang et al., 2012b). Increasing studies have focused on the neuroprotective effect of geniposide in brain diseases, especially neurodegenerative disorders. Its protective effect from memory impairment and the normalization of objection recognition has been shown in animal behavioral experiments (Gao et al., 2014; Lv et al., 2014). Using a high-throughput screen for GLP-1R agonists, geniposide was identified as an agonist for GLP-1R (Liu et al., 2006). It has been shown that the activation of GLP-1R by geniposide induces neurotrophic and neuroprotective effects in cells (Liu et al., 2009, 2012). However, the mechanisms underlying these effects have not been definitively identified. The aim of present review is to summarize a Chinese herbal medicine that can ameliorate AD symptoms and to investigate the cell and molecular mechanisms underlying its therapeutic efficacy based on AD pathogenesis hypothesis by which diabetes and abnormal glucose metabolism are involved in AD.

Metabolism and pharmacokinetics

Most herbal medicines that have been used in China, Korea, and Japan are orally administered. In general, glycosides that are the main contents in herbal medicines are brought into contact with the intestinal microflora in the alimentary tract, where it is metabolized. Geniposide, an iridoid glucoside, is a major component (≥2%) in the fruits of *G. jasminoides* Ellis. Until now, pharmacological studies of geniposide have revealed key properties, including antitumor effects (Hsu et al., 1997), modulation of DNA expression (Galvez et al., 2005), treatment of pain (Gong et al., 2014), and anti-inflammatory, coloretic, and hepatoprotective effects (Chou et al., 2003; Chen et al., 2009; Liu et al., 2010; Ma et al., 2011). However, the precise mechanisms of its effects remain poorly understood. It was found that intestinal bacteria in animals can transform geniposide into its aglycone genipin or other metabolites (Figure 1; Akao et al., 1994; Chen et al., 2008). Ten metabolites (G1–G10) involved in the metabolic processes were identified. It is interesting that all the metabolites detected were produced from the genipin or its ring-opened derivatives rather than the geniposide itself. It revealed that, when geniposide was orally administered, geniposide was first hydrolyzed to genipin by β-glucosidases. After the deglycosylation of geniposide in the liver or intestine, genipin would undergo redox or phase II metabolism immediately (Han et al., 2011). The metabolism of geniposide *in vivo* undergoes the following pathway: it is hydrolyzed first to produce the intermediate aglycone (genipin), which quickly conjugates with

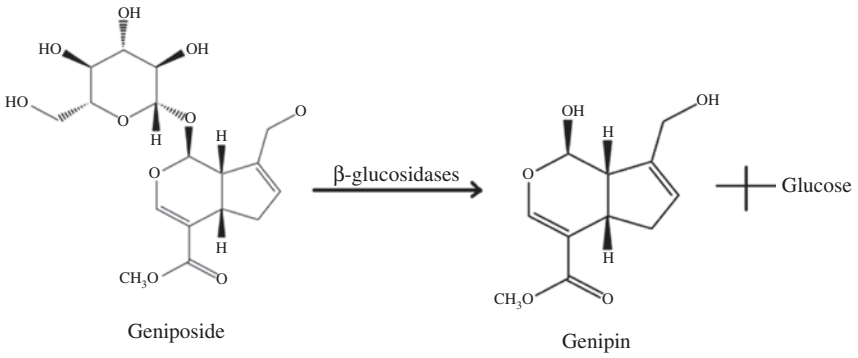

Figure 1: Mechanism of transforming geniposide into genipin.

glucuronic acid as the predominant metabolite, followed by a series of further metabolic reactions.

Previous studies had reported the pharmacokinetics of geniposide after peroral administration and intravenous (i.v.) administration in mice (Ueno et al., 2001; Ye et al., 2006; Hou et al., 2008). A more detailed information of the bioavailability and tissue distribution of geniposide is still lacking. Recently, studies on the pharmacokinetics, bioavailability, and tissue distribution of geniposide had been carried out (Sun et al., 2012; Wang et al., 2014). The major pharmacokinetic parameters of geniposide in rat plasma after oral administration are shown in Table 1 (Yu et al., 2013). Compared with the i.v. administration, the $t_{1/2}$ was prolonged after the oral administration of geniposide. In addition, the $AUC_{0\to\infty}$ value of geniposide was 6.99±1.27 h μg/ml after an i.v. administration of 10 mg/kg geniposide. After the oral administration of geniposide, the absolute oral bioavailability (%F) of geniposide was calculated as 9.67%, and the $AUC_{0\to4\ h}$ values in tissues were in the order of kidney>spleen>liver>heart>lung> brain (Yu et al., 2013).

Geniposide is widely used in Chinese medicine as a neuroprotection agent (Liu et al., 2009; Wu et al., 2009). The pharmacokinetic studies of geniposide and its increased absorption in the brain by the terpene borneol have been published (see Table 2 for details on the pharmacokinetic parameters; Yu et al., 2013). The results also demonstrated that borneol markedly facilitated the delivery of geniposide to the hippocampus. Therefore, the region-specific effect of borneol on the blood-brain barrier (BBB) might be a new strategy for the treatment of central nervous system disorders.

To better understand the pharmacokinetics of geniposide, its aglycone genipin was administered i.v. and orally. When genipin was given as an i.v. bolus, genipin levels declined rapidly and genipin sulfate emerged instantaneously, indicating that a very rapid hepatic sulfation had occurred (Hou et al., 2008). Further research needs to be performed on other similar iridoid compounds contained in various medicinal herbs to obtain a more comprehensive view of their pharmacological mechanism and metabolic fates.

Table 1: Pharmacokinetic parameters of geniposide in plasma after oral administration.

Parameters	Geniposide (40.65 mg/kg) Zhi-Zi-Hou-Pu decoction	Geniposide (100 mg/kg)
t_{max} (min)	0.79±0.19	0.5±0.03
C_{max} (μg/ml)	1.29±0.16	1.40±0.24
$t_{1/2}$ (h)	2.67±0.56	3.55±0.69
$AUC_{0-\infty}$ (h μg/ml)	5.07±1.07	6.76±1.23

Yu et al., 2013.

Molecular pathways

GLP-1R belongs to the class B family of G protein-coupled receptors. A large number of studies have shown that GLP-1 functions through its receptor to regulate insulin secretion and glucose metabolism and is therefore an important strategy in the treatment of T2DM (Shao et al., 2010; Burmeister et al., 2012). GLP-1, just like insulin and insulin-like growth factor-I, activates second messenger signaling pathways that are commonly linked to growth factor signaling (Hölscher, 2014a). Geniposide is structurally unrelated to insulin and binds to GLP-1R, thereby circumventing insulin signaling impairment. After binding to GLP-1R, it activates signaling pathways that converge with the insulin signaling pathway and facilitates insulin signaling. It was found that geniposide, with the activation of GLP-1R, induced insulin secretion in a dose-dependent manner and showed neurotrophic properties

Table 2: Pharmacokinetic parameters of geniposide in brain regions after i.v. administration.

Parameters	Cortex	Hippocampus	Hypothalamus	Striatum
t_{max} (min)	24.00±8.94	20.00±0.00	20.00±0.00	20.00±0.00
C_{max} (μg/ml)	565.80±234.21	134.87±49.00	133.13±97.76	150.46±63.02
$t_{1/2}$ (h)	1.84±0.80	2.62±2.03	1.69±1.34	2.12±0.75
$AUC_{0-\infty}$ (h μg/ml)	796.67±240.00	400±240.00	298.33±96.17	441.67±109.17
$MRT_{0-\infty}$ (h)	2.04±0.77	3.85±2.79	2.69±1.43	3.25±0.85

Yu et al., 2013.

by stimulating cAMP production. Furthermore, the phosphatidylinositol 3-kinase (PI3K) signaling pathway and the mitogen-activated protein kinases (MAPK) pathway are involved in the neuroprotection of geniposide against oxidative damage in PC12 cells and SH-SY5Y cells (Liu et al., 2007, 2012; Guo et al., 2012; Sharma et al., 2013). The activities of geniposide in neurons include an increased expression of genes that are linked to cell growth and repair, inhibition of apoptosis, and reduction of inflammatory responses (see Figure 2 for details on the underlying molecular mechanism).

Possible neuroprotective mechanisms in AD

Geniposide reduces the levels of Aβ

Plaques that are composed of aggregated Aβ ($Aβ_{1-42}$–$Aβ_{1-40}$) are a characteristic hallmark of AD. Aβ is a peptide fragment, mostly 40–42 amino acids in length, which is cleaved from the APP by β-secretases and γ-secretases (Thinakaran and Koo, 2008). Pimplikar (2009) summarized many avatars of the amyloid hypothesis in a review. It was originally proposed that increased levels of Aβ resulted in plaque formation, which caused AD. Subsequent observations that familial APP mutations increase $Aβ_{42}$ generation led to a proposal that it is the increased level of $Aβ_{42}$ peptide that causes AD. Another variation on the theme is that the absolute levels of $Aβ_{42}$ are less important than the ratio of $Aβ_{42/40}$ in causing AD (Pimplikar, 2009). Others propose that amyloid is not instrumental in the development of AD at all (Morris et al., 2014). However, the currently most favored idea is that Aβ forms soluble oligomers, which are pathogenic in nature and cause AD (Selkoe, 2008).

Aβ aggregation into soluble oligomers is believed to be the main toxic species and the causative agent underlying the pathological mechanism for AD, aggregating and accumulating within and around neurons, causing cognitive dysfunction, including memory loss (Selkoe, 2008; Rakez and Cristian, 2013). There is also evidence that the increased level of Aβ depresses excitatory synapses and reduces neuronal activity, and in contrast to the pathological accumulation in normal brain, Aβ is produced at lower concentration (Kamenetz et al., 2003; Parihar and Brewer, 2010). As a downstream effect, τ pathology in AD associated with the cognitive impairment was initiated.

It is not surprising that the metabolism of Aβ has become an important therapeutic target in AD research. Understanding the processing and secretion of APP and its relationship to Aβ opens a window to develop compounds that prevent the production of Aβ by affecting the cleavage of APP or the aggregation, clearance, or toxicity of Aβ (Sabbagh et al., 2000).

The iridoid glucosides extracted from *G. jasminoides* showed a potential improvement of short-term learning/memory capacities in human $Aβ_{42}$-expressing transgenic flies (Yu et al., 2009). It suggests that the component of *G. jasminoides* might have a potential protective effect against neurodegenerative processes in AD. Preincubation with geniposide prevented primary cultured cortical neurons from $Aβ_{1-42}$-induced injury. Geniposide also induced the expression of insulin-degrading enzyme (IDE), a major degrading protease of Aβ, in a dose-dependent manner (Yin et al., 2012). These findings indicate that geniposide activates GLP-1Rs, which then protects against Aβ-induced neurotoxicity by the regulation of the expression of IDE in cortical neurons. The cultured hippocampal neurons had significantly degenerated after treatment with $Aβ_{25-35}$, but the degeneration did not occur to the same extent in the presence of genipin (Yamazaki et al., 2001b). One study found that genipin suppressed apoptosis in cultured cells via the inhibition of caspase activation and mitochondrial function (Yamamoto et al., 2000). Furthermore, strong evidence suggests that geniposide regulates the expression of apoptosis-related proteins via the MAPK signaling pathway, thereby overcoming the toxicity of Aβ (Liu et al., 2007). All of those studies indicate

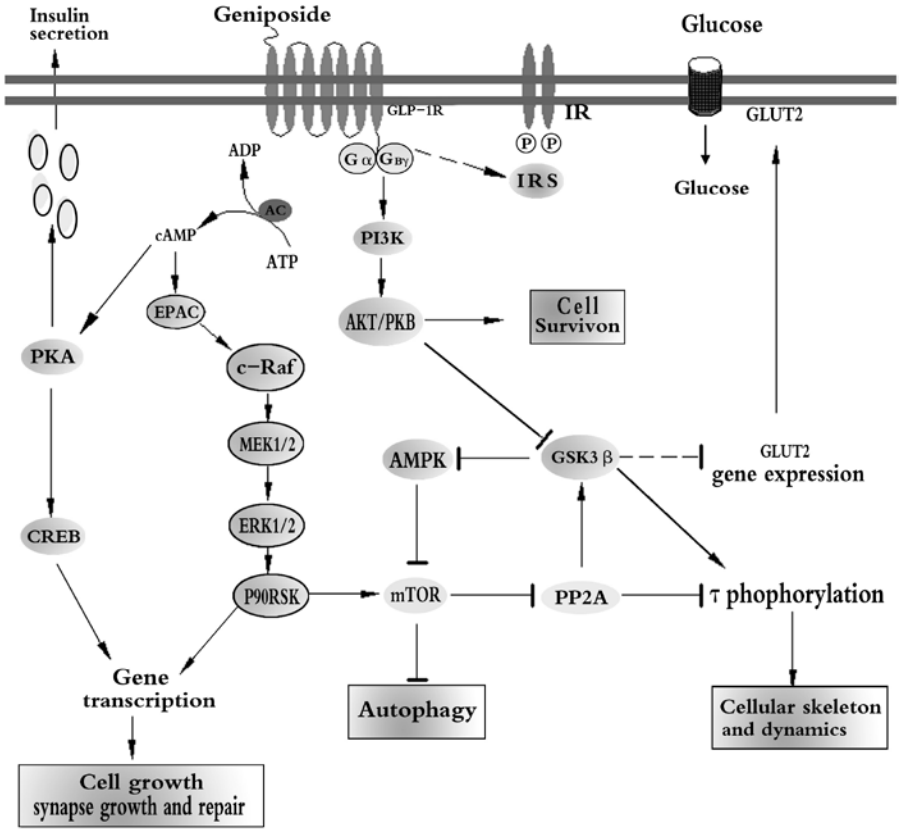

Figure 2: Overview of the main pathways induced by geniposide in neurons.
Geniposide activated GLP-1R in a manner similar to GLP-1. GLP-1R is a member of a different class of receptors compared with insulin receptor (IR). The activation of GLP-1R activates adenylyl cyclase and increases cAMP levels (Green et al., 2004), which stimulates PKA and enhances the transcription of IR substrate (IRS) 2 (Broca et al., 2009). By this pathway, it can link with the signaling pathway of IR. The phosphorylation of PKA and other downstream kinases is related to insulin secretion and growth factor signaling. An increase of PI3K levels via the G protein activation can activate the following pathways: (1) The MAPK pathway activates gene expression, which controls the expression of peptides that are required for cell growth and repair in neuronal cells (Perry et al., 2003), and also ERK1/2 and PI3K, which also activate the MAPK pathway (Sharma et al., 2013). (2) Geniposide also suppresses the induction of apoptosis. This pathway involves the stimulation of PI3K binding to IRS and G protein, and the activation of PI3K and protein kinase B/Akt, which suppresses the induction of apoptosis and thereby protects neurons (Liu et al., 2012). (3) The activation of GSK3 modifies the cellular skeleton and dynamics by mediating the phosphorylation levels of τ protein, modulating the cleavage of APP, and improving learning and memory formation (Eldar-Finkelman et al., 1999; Gao et al., 2011, 2014). As well, AMPK inhibits the mammalian target of rapamycin complex resulting in autophagy stimulation. This pathway also suppresses glucose transporter 2 (GLUT-2) and GLUT-4 gene expression. Traditionally, insulin is associated with its blood glucose-lowering activity. This is achieved by activating a glucose uptake transporter (e.g., GLUT-4). This function is only one of many of the IR and GLP-1R (Perry and Greig, 2005; Hölscher, 2011, 2014a).

that geniposide and genipin are the potential candidates for preventing the development of AD.

Inhibition of τ phosphorylation by geniposide

Hyperphosphorylated τ protein was identified as the major component of NFTs, which are known to be a key pathological feature of AD (Grundke-Iqbal et al., 1986). τ protein is a highly soluble microtubule-associated protein found in the axonal compartment of the neuron. Its primary function is involved in microtubule stabilization, axonal transport, homeostasis, and synaptic function (Drubin and Kirschner, 1986; Terwel et al., 2002). The integrity of the microtubules is maintained by the phosphorylation state of τ, which is regulated by many phosphatases and kinases (Avila et al., 2004; Hashiguchi and Hashiguchi, 2013). Glycogen synthase kinase 3 (GSK3) has been identified as the key kinase responsible for the hyperphosphorylation of τ in AD (Flaherty et al., 2000; Hooper et al., 2008; Llorens-Martín et al., 2014). When τ protein is phosphorylated, it results in the disassembling of microtubules and can aggregate abnormally when

hyperphosphorylated to form NFTs. Once the aggregation into NFTs occurs, τ loses the function of connecting to tubulin and can no longer play a role in the microtubule assembly. Thus, the inhibition of pathological hyperphosphorylation of τ may be a therapeutic target for AD (Iqbal et al., 2010; Ma et al., 2014).

Various animal models have enabled the identification and characterization of key cellular processes that promote apoptosis in tauopathy, including synapse loss, impaired axonal transport, overstabilization of filamentous actin, mitochondrial dysfunction, and aberrant cell cycle activation in postmitotic neurons (Frost et al., 2015).

Identifying the causes of abnormal τ phosphorylation and aggregation is a major target for the development of therapeutic interventions for tauopathies and has been the focus of much research, including AD (Götz et al., 2012; Medina and Avila, 2014). Current strategies include decreasing τ aggregation, blocking abnormal τ phosphorylation, or stopping the spread of τ pathology through the brain. Our previous study (Gao et al., 2014) showed that geniposide could greatly reverse τ hyperphosphorylation and the paired helical filament-like structures induced by STZ. Furthermore, we also showed that the neuroprotective effect of geniposide was blocked by wortmannin, a PI3K inhibitor. This indicates that the signaling of PI3K/GSK3 is involved in the phosphor-τ decrease effect of geniposide.

Attenuation of mitochondrial oxidative stress by geniposide

The multiple pathogenic mechanisms contributing to the pathology of AD include an increase of reactive oxygen species production, mitochondrial dysfunction, and apoptosis due to the impairment of mitochondrial Ca^{2+} handling ability, altered Ca^{2+} homeostasis, increased mitochondrial permeability transition pore opening, and promotion of cytochrome *c* release (Godoy et al., 2014). Studies using transgenic mice demonstrated alterations in mitochondrial enzymes in the AD brain (Piaceri et al., 2012). It has been shown that one of the neurotoxic mechanisms of Aβ peptides is increasing oxidative stress in cultural neurons (Lee et al., 2010). Moreover, the enhancement of the oxidative stress by the *in vivo* depletion of vitamins has been shown to result in an increased amount of Aβ by the inhibition of its clearance from the brain (Habib et al., 2010). These suggest that oxidative stress, either by itself or as part of a 'two-step process', causes neuronal dysfunction and eventually AD (Ciron et al., 2012). Many treatment strategies have been focused on preserving mitochondrial function in AD. The underlying mechanism of action seems to be related to the prevention of mitochondrial Ca^{2+} overload, and the modulation of the fusion-fission process, thereby arresting mitochondrial dysfunction (Dinamarca et al., 2008). The induction of endogenous antioxidative proteins seems to be a reasonable strategy for delaying the progression of cell injury.

It has been shown that the intragastric administration of geniposide significantly reduces oxidative stress and increases the mitochondrial membrane potential and activity of cytochrome *c* oxidase in addition to improving learning and memory in APP/PS1 mice (Lv et al., 2014). Genipin was evaluated for its ability to inhibit oxidative effects in rat brain homogenate initiated by an Fe^{2+}/ascorbate system. It inhibited the generation of malondialdehyde, which reacts with *N*-methyl-2-phenylindole. Besides, genipin is a specific hydroxyl radical scavenger (Koo et al., 2004). Geniposide induced glutathione *S*-transferase (GST) activity and the expression of GST M1 and M2 acting in primary cultured rat hepatocytes through the expression of MEK-1 signaling proteins and the activation of Ras/Raf/MEK-1 signaling pathway. GSTs are a family of dimeric enzymes, which is responsible for the metabolism of a broad range of xenobiotics (Kuo et al., 2005).

Geniposide activated GLP-1R, leading to an increase in intracellular cAMP. Furthermore, geniposide could increase the expression of HO-1 and resist the oxidative damage induced by H_2O_2 and 3-morpholinosydnonimine hydrochloride (SIN-1) in PC12 cells by activating the MAPK-p90RSK, PI3K/Akt-Nrf2, and protein kinase A (PKA)-cAMP-response element binding protein signaling pathways (Liu et al., 2007, 2009; Yin et al., 2010a,b). Pretreatment with geniposide markedly improved the viability of cells and regulated the expression of apoptotic protein involved in mitochondrial-mediated apoptosis in PC12 cells induced by $CoCl_2$. The results demonstrated that geniposide had a significant influence on the mitochondrial function, which was damaged by oxidative stress induced by $CoCl_2$ (Guo et al., 2009). Genipin has an ability to induce neurite outgrowth through the activation of several protein kinases including extracellular signal-regulated kinase (ERK) and the activation of nitric oxide (NO) synthase (NOS) in PC12h cells. Studies also have shown that the NO/cGMP pathway suppresses 6-OHDA-induced apoptosis in PC12 cells by inhibiting the mitochondrial cytochrome *c* release and caspase-3 and -9 activation via PKG/PI3K/Akt-dependent Bad phosphorylation (Ha et al., 2003; Matsumi et al., 2008).

Inhibition of ER stress by geniposide

The ER is a membranous cell organelle in which key cell functions take place, such as protein synthesis, and folding and transport of translocating and integrating proteins (secretory and membrane proteins), lipid biosynthesis, and maintaining calcium homeostasis (Fagone and Jackowski, 2009; Sammels et al., 2010). The disturbance in ER function via the accumulation of unfolded and deficiently modified proteins and the release of ER luminal Ca^{2+} into the cytoplasm results in ER stress; chronic ER stress emerges as a key factor driving neuronal degeneration and cognitive impairment beyond cell death, a late event on disease progression, which has been linked to a variety of age-related neurodegenerative diseases, such as AD and Parkinson's disease (Antero et al., 2009; Salminen et al., 2010; Torres et al., 2014). A large body of evidence indicates that the ER stress response is localized to dendrites. This heterogeneity of the ER network may be related to axonal degeneration and synaptic loss in neurons, particularly in the case of redox-based dysfunctions, emphasizing a role for ER stress in neuronal degeneration (Raff et al., 2002; Murakami et al., 2007; Banhegyi et al., 2008). Mostly, the reduction of amyloid plaques is correlated with attenuated ER stress and vice versa. It is revealed that treadmill exercise prevented PS2 mutation-induced memory impairment and reduced $A\beta_{42}$ deposition and ER stress through the inhibition of β-secretase in the cortex and/or hippocampus of aged PS2 mutant mice. It showed that APP processing and phosphorylation of τ might be influenced by ER stress signaling (Endres and Reinhardt, 2013). Therefore, elucidating ER stress in AD might help turning the scale in therapeutic considerations or for the evolvement of new highly diagnostic biomarkers.

Currently, no evidence exists that geniposide and genipin suppress ER stress that is induced by Aβ. However, several studies (Yamazaki et al., 2009; Tanaka et al., 2009) show the protective effects of genipin on cytotoxicity induced in Neuro2a cells by tunicamycin, an ER stress inducer. Genipin dramatically rescued the cells against tunicamycin-induced cell death. In addition, genipin suppressed ER stress-induced up-regulation of glucose-regulated protein of 78 kDa (also known as Bip) and CCAAT/enhancer-binding protein homologous protein (also known as growth arrest and DNA damage-inducible gene 153) also suppressed the activation of caspases 3/7 and 12. Another study examined the potential regulatory effects of geniposide on hepatic dyslipidemia and its related mechanisms *in vitro* and *in vivo*. The authors found that geniposide inhibited palmitate-induced ER stress, reducing hepatic lipid accumulation through the secretion of apolipoprotein B and associated triglycerides and cholesterol in human HepG2 hepatocytes (Lee et al., 2013). Oral administration of geniposide was also reduced in middle cerebral artery occlusion rat model (Pan et al., 2014).

Inhibition of chronic inflammation in AD by geniposide

Inflammation is a complex molecular and cellular defense mechanism in response to stress, injury, and infection. Although the etiologic mechanisms of AD are poorly understood, more recently, an analysis of human brain AD samples has shown highly expressed inflammatory cytokines and an up-regulation in inflammatory genes during the early stages of AD (Hollingworth et al., 2011; Sudduth et al., 2013). During neurodegenerative disease development, microglia and other cell types, including cytokines, are activated in response to misfolded proteins in the brain, also participate in the active immune defense, and are particularly important in regulating tissue homeostasis and in preserving the structural and functional characteristics of the brain (Heneka et al., 2014; Fakhoury, 2015). McGeer et al. (1988) demonstrated the activation of microglial cells and astroglial cells in close proximity to the damaged or dying neurons. The accumulation of glial cells around plaques along with the strong up-regulation of inflammatory markers has been taken as evidence that glial cell proliferation is a key element of the disease process. This is supported by several *in vivo* studies using markers for proliferating cells in transgenic mice. Elevated levels of inflammatory cytokines, tumor necrosis factor (TNF)-α, interferon-γ, and interleukins (ILs), particularly IL-1β and IL-18, are found in the brain, near the Aβ plaques, in AD patients and transgenic mice (Johnston et al., 2011; Rubio-Perez and Morillas-Ruiz, 2012).

Modern medical practice has proven that some of Chinese herbal medicine can have anti-inflammatory effects in patients. Gardenia fruit extracts (GRE) contain acute anti-inflammatory activities, and geniposide and genipin are possibly responsible for those activities of GRE (Koo et al., 2006). In the treatment of various peripheral inflammation, genipin performs its anti-inflammatory activity through the suppression of both NO production and cyclooxygenase expression. Geniposide also decreases serum lipopolysaccharide level and inhibits cytokine (TNF-α and IL-6) release in mice (Zhu et al., 2005; Zheng et al., 2010). Several studies demonstrated that geniposide exerted anti-inflammatory effects by interfering with the expression of Toll-like receptor 4, which subsequently inhibited the downstream nuclear factor-κB

(NF-κB) and MAPK signaling pathways and the release of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 (Wang et al., 2012a; Huang et al., 2013; Song et al., 2014).

Aβ acts as a microglia activator in cell culture studies (Meda et al., 1995). Genipin significantly repressed NO release from microglia that have been stimulated with Aβ. Nevertheless, more work is required on identifying target molecules of genipin involved in signaling pathways modulating the microglial inflammatory response. The receptor for advanced glycation end products (RAGE), an immunoglobulin-like cell surface receptor, is also known to be an important cellular cofactor for Aβ-mediated cellular perturbation (Yan et al., 2012). The mechanisms by which Aβ mediates the activation of microglia and astrocytes remain to be elucidated. It appears that there is an important role for RAGE-mediated signaling in the microglial activation and neuronal dysfunction. RAGE triggers the generation of proinflammatory cytokines at the BBB (Leclerc et al., 2010). Further, RAGE-dependent signaling in microglia stimulates inflammatory responses and processes that exacerbate neuronal damage, ultimately impairing neuronal function in the cultured cells taken from AD and AD transgenic mice (Lue et al., 2001; Yan et al., 2009; Fang et al., 2010). Recent studies demonstrate that geniposide significantly blocks RAGE-dependent signaling (activation of ERK and NF-κB), with Aβ induced along with the production of TNF-α and IL-1β. Notably, based on the data from coimmunoprecipitation assay, they infer that geniposide exerts protective effects on the Aβ-induced inflammatory response through blocking Aβ binding to RAGE and suppressing the RAGE-mediated signaling pathway (Lv et al., 2015). Taking those together, RAGE may be a target for a novel AD therapy.

Neurite outgrowth promoted by geniposide

Nerve growth factor (NGF), a neurotrophin, plays a trophic role both during development and in adulthood and activates the tropomyosin kinase receptor A (TrkA)-Ras-ERK signaling pathway by interacting with the specific receptor TrkA (Patapoutian and Reichardt, 2001; Huang and Reichard, 2003; Aloe et al., 2012). Also, NGF elicits its neuritogenic effect through the activation of neuronal NOS (nNOS) followed by the activation of the NO-cGMP-PKG signaling pathway (Hartikka and Hefti, 1988). Further studies on NGF deficit-induced neurodegeneration in transgenic mice demonstrated also a novel causal link between neurotrophic signaling deficits and AD (Cattaneo and Calissano, 2012). There are growth cones at the free terminals of long neurites in PC12 cells. Neurites induced by genipin generally seemed to be more branched than those induced by NGF. The addition of ERK kinase inhibitors could almost completely abolish the neurite induction. A neuritogenic effect of genipin in PC12h cells was also inhibited by the NOS inhibitor, NO scavenger, and protein kinase C (cGMP-dependent kinase) inhibitor (Yamazaki et al., 1996, 2001a, 2004). These findings suggest that NO production followed by the cGMP-mediated stimulation of the MAPK cascade is implicated in the neuritogenesis by genipin in PC12 cells. Further, it seems that geniposide and genipin promote neuronal development via different molecular mechanisms. Normal PC12 cells have no nNOS, although PC12 and PC12h cells share the same origin. Treatment with geniposide promoted cellular growth, but treatment with genipin did not (Yamazaki et al., 2006). This indicates that nNOS is the common target of geniposide and genipin and that geniposide possesses additional therapeutic targets. Perry et al. (2002) were the first to describe the effects of GLP-1 and its long-acting analogue, exendin-4, on neuronal proliferation and differentiation and on the metabolism of two neuronal proteins in PC12 cells, which had been shown to express GLP-1R. This study demonstrated that GLP-1 and exendin-4 induced neurite outgrowth in a manner being similar to NGF. A significant increase on the GAP-43 protein level in parallel with neurite outgrowth was observed after the treatment with geniposide. The data also demonstrate that geniposide induces the neuronal differentiation of PC12 cells via the MAPK pathway (Liu et al., 2006). Therefore, geniposide has neuroprotective effects due to the activation of GLP-1R in cells without nNOS. It is speculated that there is a correlation between the effect of the two drugs and the structural difference (Liu et al., 2012).

Conclusion

As a receptor agonist of GLP-1R, geniposide is a novel drug candidate for the treatment of AD because of its multiple effects in neuroprotection. As the world's aging population continues to increase and the treatment of AD is still a worldwide problem, the therapeutic potential of geniposide, which may delay the onset of age-related disorders, is highly desirable. The molecular mechanism and therapeutic targets of geniposide are not completely understood and require further research. Geniposide is water soluble and orally active and also can cross the BBB. It is easy to administer and has been shown to be safe to take. In the present review, we describe the possible mechanisms of the neuroprotective properties of geniposide and genipin:

inhibiting Aβ toxicity, oxidative stress, mitochondrial damage, ER stress, inflammation, and τ phosphorylation. In summary, the Chinese traditional medicine geniposide may be used as a novel treatment of sporadic AD and other diseases. Clinical trials in AD patients are warranted to test this hypothesis.

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