Neuroprotective effects of geniposide from Alzheimer's disease pathology

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Abstract

A growing body of evidence have linked two of the most common aged-related diseases, type 2 diabetes mellitus (T2DM) and Alzheimer 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 (GLP-1R) which is a newer drug class to treat T2DM, Geniposide shows clear effects in inhibiting pathological processes underlying AD, such as and promoting neurite outgrowth. In the present article, we review possible molecular mechanisms of geniposide to protect the brain from pathologic damages underlying AD: reducing amyloid plaques, inhibiting tau 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.

Key words: Alzheimer’s disease, geniposide, amyloid-β, neurofibrillary tangles, oxidative stress, inflammatation, type 2 diabetes mellitus, glucagon like peptide receptor, neuroprotection, tau protein

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of progressive cognitive decline in the aged population. The characteristic pathological hallmarks are abundance of two abnormal aggregated proteins in brain tissue: neurofibrillary tangles (NFTs) composed mainly of the microtubule-associated protein tau 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 (Meares et al., 2011; Stalder et al., 1999).

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). Present medications approved by the FDA 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 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 T2DM had been identified as a risk factor for AD, It is possible to develop drugs that can treat T2DM to also treat AD. 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 (Biessels et al., 2006; Haan et al., 2006; Ristow et al., 2004). 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 (Li et al., 2007; Nelson et al., 2005).

There are currently clinical trials ongoing that test the effectiveness of 'antidiabetic' drugs in AD patients. We are aware of two ongoing pilot studies of GLP-1 analogs 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 Alzheimer's Disease (ELAD) 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 analogs have shown considerable results in vivo and vitro (Hölscher, 2014a; Wang et al., 2010). The GLP-1 analog 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 was 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 analogues lixisenatide also had these impressive neuroprotective effects (McClean et al., 2014b). A study characterized the effects of another GLP-1 receptor agonist, exendin-4, on stress-induced toxicity in neuronal cultures and on Aβ and tau 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 treatment of hepatic disease, inflammation disorders, contusions and brain disorders (Wang et al., 1992; Chen et al., 2010; Wang et al., 2012). Increasing studies have focused on the neuroprotective effect of geniposide in brain diseases, especially neurodegenerative disorders. Its protective effect from memory impairment and normalisation 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-1 receptor agonists, geniposide was identified as an agonist for the GLP-1 receptor (Liu et al., 2006). It has been shown that the activation of the GLP-1 receptor by geniposide induces neurotrophic and neuroprotective effect in cells (Liu et al., 2009, 2012). But 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 is involved in AD.

2. Metabolism and pharmacokinetics

Most herbal medicines which have been used in China, Korea, and Japan are orally administered. In general, glycosides which are the main contents in herbal medicines, are brought into contact with the intestinal microflora in the alimentary tract, where it is metbolised. Geniposide, an iridoid glucoside, is a major component ($\geq$ 2%) in the fruits of Gardenia 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), anti-inflammatory, coloretic and hepatoprotective effects (Chen et al., 2009;

Liu et al., 2010; Chou et al., 2003; Ma et al., 2011). However, the precise mechanisms of its effects remains poorly understood. It was found that intestinal bacteria in animals can transform geniposide into its aglycone genipin or other metabolites (Fig. 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 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 glucuronic acid as the predominant metabolite, followed a series of further metabolic reactions.

Previous studies had reported the pharmacokinetics of geniposide after peroral administration and intravenous (i.v.) administration in mice (Hou et al., 2008; Ueno et al., 2001; Ye et al., 2006). 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 pharmacokinetics 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 oral administration of geniposide. In addition, The AUC0 $\rightarrow\infty$ values of geniposide were 6.99 ± 1.27 h · μg/ml following i.v. administration of 10 mg/kg of geniposide. After oral administration of geniposide, the absolute oral bioavailability (%F) of geniposide was calculated as 9.67%, the AUC0 $\rightarrow$4h 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). 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 (CNS) disorders.

In order to better understand the pharmacokinetics of geniposide, its aglycone genipin was administered intravenously and orally. When genipin was given as an intravenous 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.

3. Molecular pathways

The GLP-1 receptor belongs to the class B family of G protein-coupled receptors (GPCRs). 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 (Burmeister et al., 2012; Shao et al., 2010). GLP-1 just like insulin and IGF-1 activates second messenger signaling pathways that are commonly linked to growth factor signaling (Holscher, 2014a). Geniposide is structurally unrelated to insulin and binds to GLP-1R, thereby circumventing insulin-signaling impairment. After binding to the GLP-1R, it activates signaling pathways that converge with the insulin-signaling pathway and facilitate insulin signaling. It was found that geniposide, with the activation of GLP-1 receptor, induced insulin secretion in a dose-dependent manner and showed neurotrophic properties by stimulating cAMP production. Furthermore, the phosphatidylinositol 3-kinase (PI3K) signaling pathway and mitogen-activated protein kinases (MAPK) pathway are involved in the neuroprotection of geniposide against oxidative damage in PC12 cells and in SH-SY5Y cells (Liu et al., 2007; Liu et al., 2012; Guo et al.,

2012; Sharma et al., 2013). The activities of geniposide in neurons include increased expression of genes that are linked to cell growth and repair, inhibition of apoptosis and reduction of inflammatory responses (see Fig. 2 for details on the underlying molecular mechanism).

4. Possible neuroprotective mechanisms in AD

4.1 Geniposide reduces levels of Aβ

Plaques which are composed of aggregated Aβ ($A\beta_{1-42}$ - $A\beta_{1-40}$) 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 et al., 2008). Pimplikar (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 $A\beta_{42}$ generation led to a proposal that it is the increased levels of $A\beta_{42}$ peptide that causes AD. Another variation on the theme is that the absolute levels of $A\beta_{42}$ are less important than the ratio of $A\beta_{42/40}$ 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 are believed to be the main toxic species and the causative agent underlying the pathological mechanism for AD, aggregating and accumulating within and around neurons, cause 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, tau pathology in AD associated with the cognitive impairment was initiated.

It’s 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 Gardenia Jasminoides showed potential improvement of short-term learning/memory capacities in human $A\beta_{42}$ –expressing transgenic flies (Yu et al., 2009). It suggests that the component of Gardenia Jasminoides might have potential protective effect against neurodegenerative processes in AD. Pre-incubation with geniposide prevented primary cultured cortical neurons from $A\beta_{1\text{-}42}$-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-1 receptors, which then protects against Aβ-induced neurotoxicity by regulation of the expression of IDE in cortical neurons. The cultured hippocampal neurons had significantly degenerated after treatment with $A\beta_{25\text{-}35,}$ but the degeneration did not occur to the same extent in the presence of genipin (Yamazaki et al., 2001). One study found that genipin suppressed apoptosis in cultured cells via inhibition of caspase activation and mitochondrial function (Yamamoto et al., 2000). Furthermore, strong evidence suggests that geniposide regulates 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 that geniposide and genipin are potential candidates for preventing the development of AD.

4.2 Inhibition of Tau phosphorylation by geniposide

Hyperphosphorylated tau protein was identified as the major component of neurofibrillary tangles, which are known to be a key pathological feature of AD (Grundke-Iqbal et al., 1986). Tau 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). Integrity of the microtubules is maintained by the phosphorylation state of tau, which is regulated by many phosphatases and kinases (Avila et al., 2004; Hashiguchi et al., 2013). GSK-3 has been identified as the key kinase responsible for the hyperphosphorylation of tau in AD (Flaherty et al., 2000; Hooper et al., 2008; Llorens-Martín et al., 2014). When tau protein is phosphorylated, it results in the disassembling of microtubules and can aggregate abnormally when hyperphosphorylated to form neurofirillary tangles. Once the aggregation into neurofirillary tangles occurs, tau loses the function of connecting to tubulin and can no longer play a role in the microtubule assembly. Thus, inhibition of pathological hyperphosphorylation of tau may be a therapeutic target for AD (Iqbal et al., 2010; Ma et al., 2014).

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

Identifying the causes of abnormal tau 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 et al., 2014). Current strategies include decreasing tau aggregation, blocking abnormal tau phosphorylation, or stopping the spread of tau pathology through the brain. Our previous study (Gao et al., 2014) showed that geniposide could greatly reverse tau hyperphosphorylation and the paired helical filament like structures (PHFs) induced by STZ. Furthermore, we also showed that neuroprotective effect of geniposide was blocked by Wortmannin, a PI3k inhibitor. This indicates that signaling of PI3K/GSK3 is involved in the phosphor-tau decrease effect of geniposide.

4.3 Attenuation of mitochondrial oxidative stress by geniposide

The multiple pathogenic mechanisms contributing to the pathology of AD include an

increase of reactive oxygen species (ROS) 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 it clearance from the brain (Habib et al., 2012). 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 modulation of the fusion-fission process, thereby arresting mitochondrial dysfunction (Dinamarca et al., 2008). Induction of endogenous antioxidative proteins seems to be a reasonable strategy for delaying the progression of cell injury.

It has been shown that intra-gastric 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 GST 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. Glutathione S-transferases (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 the GLP-1 receptor, 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 PKA-CREB (cAMP-response element binding protein) signaling pathways (Liu et al., 2009; Liu et al., 2007;Yin et al., 2010; Yin et al., 2010). Pretreatment with geniposide markedly improved the cells' viability 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 activation of several protein kinases including extracellular signal-regulated kinase (ERK) and activation of nitric oxide 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, caspase-3 and -9 activation via PKG/PI3K/Akt-dependent Bad phosphorylation (Matsumi et al., 2008; Ha et al., 2003)

4.4 Inhibition of ER stress by geniposide

The endoplasmatic reticulum (ER) is a membranous cell organelle in which key cell functions takes 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 et al., 2009; Sammels et al., 2010). Disturbance in ER function via the accumulation of unfolded and deficiently modified proteins, and 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 (PD) (Antero et al., 2009; Salminen et

al., 2010; Torres et al., 2014). A large body of evidence indicates that the ER stress response is localised 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, reduction of amyloid plaques is correlated with attenuated ER-stress and vice versa. It is revealed that treadmill exercise (TE) prevented PS2 mutation-induced memory impairment and reduced Aβ-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 tau might be influenced by ER-stress signaling (Kristina and Sven, 2013). Therefore, elucidating ER-stress in AD might help turning the scale in therapeutic considerations or for evolvement of new highly diagnostic biomarkers.

Currently, no evidence existas that geniposide and genipin suppresses ER stress that is induced by Aβ. However, several studies (Tanaka et al., 2009; Masayuki et al., 2009) show the protective effects of genipin on cytotoxicity induced in Neuro2a cells by tunicamycin (TM), an ER stress inducer. Genipin dramatically rescued the cells against TM-induced cell death. In addition, genipin suppressed ER stress-induced upregulation of glucose-regulated protein of 78 kDa (GRP78, also known as Bip) and CCAAT/enhancer-binding protein(C/EBP) homologous protein (CHOP, also known as growth arrest and DNA damage-inducible gene 153(GADD153)), also suppressed the activation of caspase-3/7 and caspase 12. Another studyexamined the potential regulatory effects of geniposide on hepatic dyslipidemia and its related mechanisms *in vitro* and *in vivo*. The authors found geniposide inhibited palmitate-induced ER stress, reducing hepatic lipid accumulation through secretion of apolipoprotein B and associated triglycerides and cholesterol in human HepG2 hepatocytes (Lee et al., 2013). Oral administration of geniposide also reduced in middle cerebral artery occlusion rat model (Pan et al., 2014)

4.5 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, analysis of human brain AD samples has shown highly expressed inflammatory cytokines and an upregulation 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. (1998) demonstrated the activation of microglial cells and astroglial cells in close proximity to the damaged or dying neurons. The accumulation of glia cells around plaques along with strong upregulation of inflammatory markers has been taken as evidence that glia 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, TNFα, IFNγ, and interleukins, in particular 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 et al., 2012).

Modern medical practice has proved that some of Chinese herbal medicine can have anti-inflammatory effects in patients. Gardenia fruit extracts (GRE) contain acute anti-inflammatory activities, geniposide and genipin are possibly responsible for those activities of GRE (Koo et al., 2006). In treatment of various peripheral inflammation, genipin performs its anti-inflammatory activity though the suppression of both NO production and cyclooxygenase expression. Geniposide also decreases serum LPS level and inhibits cytokine (TNF-α and IL-6) release in mice (Zheng et al., 2010; Zhu et al., 2005). Several studies demonstrated that geniposide exerted anti-inflammatory effects by interfering with the expression of Toll-like receptors 4 (TLR4), which subsequently inhibited the downstream NF-κB and MAPK signaling pathways and the release of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 (Song et al., 2014; Wang et al., 2012; Huang et al., 2013).

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 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 pro-inflammatory cytokines at the blood brain barrier (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 (Yan et al., 2009; Fang et al., 2010; Lue et al., 2001). Recent studies demonstrate that geniposide significantly blocks RAGE-dependent signaling (activation of ERK and NF-κB) Aβ-induced along with the production of TNF-α and IL-1β. Notably, based on the data from co-immunoprecipitation 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). Taken those together, RAGE may be a target for a novel AD therapy.

4.6 Neurite outgrowth promoted by geniposide

Nerve growth factor (NGF), a neurotrophin, plays a trophic role both during development and in adulthood, and activates TrkA-Ras-ERK signaling pathway by interacting with the specific receptor tropomyosin kinase receptor A (TrkA) (Aloe et al., 2012; Huang et al., 2003; Patapoutian and Reichardt, 2001). Also, NGF elicits its neuritogenic effect through activation of nNOS followed by activation of 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 PC 12 cells. Neurites induced by genipin generally seemed to be more branched than those induced by NGF. 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 PKC (cGMP-dependent kinase) inhibitor (Yamazaki et al., 1996, 2001, 2004). These findings suggest that NO production followed by 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 even though PC12 cells and PC12h cells share the same origin. Treatment with geniposide promoted cellular growth, yet 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 (Perry et al., 2002) was 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 the GLP-1 receptor. This study demonstrated that GLP-1 and exendin-4 induced neurite outgrowth in a manner being similar to nerve growth factor (NGF). A significant increase on the GAP-43 protein level in parallel with neurite outgrowth was observed after 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 the GLP-1 receptor 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 ageing 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 blood-brain-barrier. 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 tau 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.

References

Akter, K., Lanza, E.A., Martin, S.A., Myronyuk, N., Rua, M., and Raffa, R.B. (2011). Diabetes and Alzheimer ' s disease: shared pathology and treatment? Br. J. Clin. Pharmacol.71 , 365 – 376.

Akao, T., Kobashi, K., Aburasa, M., (1994). Enzymic studies on the animal and intestinal bacterial metabolism of geniposide. Biol. Pharm. Bull. 17, 1573–1576.

Aloe, L., Rocco, M.L., Branching, P., Mani, L. (2012). Nerve growth factor: from the early discoveries to the potential clinical use. J. Transl. Med. 10, 239.

Alvarez, G., Munoz-Montano, J.R., Satrustegui, J., Avila, J., Bogonez, E., and Diaz-Nido, J. (1999). Lithium protects cultured neurons against β -amyloid-induced neurodegeneration. FEBS Lett. 453 , 260 – 264.

Antero, S., Anu, K., Tiina, S., Kai, K., Johanna, O. (2009). ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. Journal of Neuroinflammation. 6:41.

Avila, J., Lucas, J.J., Perez, M., Hernandez F. (2004). Role of Tau Protein in Both Physiological and Pathological Conditions. Physiol Rev. 84, 361-84.

Banhegyi, G., Mandl, J., Csala, M. (2008). Redox-based endoplasmic reticulum dysfunction in neurological diseases. J Neurochem. 107, 20-34.

Biessels, G.J., De Leeuw, F.E., Lindeboom, J., Barkhof, F & Scheltens, P. (2006). Increased cortical atrophy in patients with Alzheimer's disease and type 2 diabetes mellitus. Journal of Neurology, Neurosurgery, and Psychiatry. 77, 304–307.

Broca, C., Quoyer, J., Costes, S., Linck, N., Varrault, A., Deffayet, P.M., Bockaert, J., Dalle, S., Bertrand, G. (2009). Beta-Arrestin 1 is required for PAC1 receptor-mediated potentiation of long-lasting ERK1/2 activation by glucose in pancreatic beta-cells. J Biol Chem. 284, 4332-42.

Burmeister, M.A., Ferre, T., Ayala, J.E., King, E.M., Holt, R.M., Ayala, J.E. (2012). Acute activation of central GLP-1 receptors enhances hepatic insulin action and insulin secretion in high-fat-fed, insulin resistant mice. Am J Physiol Endocrinol Metab. 302, E334-343.

Cattaneo, A., Calissano, P. (2012). Nerve growth factor and Alzheimer's disease: new facts for an old hypothesis. Mol. Neurobiol. 46, 588–604.

Chen, C., Han, F., Zhang, Y., Lu, J., Shi, Y. (2008). Simultaneous determination of geniposide and its metabolites genipin and genipinine in culture of Aspergillus niger by HPLC. Biomed. Chromatogr. 22, 753.

Chen, Q.C., Zhang, W.Y., Kim, H., Lee, I.S., Ding, Y., Youn, U.J., Lee, S.M., Na, M., Min, B.S., Bae, K. (2010). Effects of Gardeniae Fructus extract and geniposide on promoting ligament cell proliferation and collagen synthesis. Phytother Res Suppl 1, S1–S5.

Chen, Q.C., Zhang, W.Y., Youn, U., Kim, H., Lee, I., Jung, H.J., Na, M., Min, B.S., Bae, K. (2009). Iridoid glycosides from Gardeniae fructus for treatment of ankle sprain. Phytochemistry. 70, 779.

Chou, C.C., Pan, S.L., Teng, C.M., Guh, J.H. (2003). Pharmacological evaluation of several major ingredients of Chinese herbal medicines in human hepatoma

Hep3B cells. Eur. J. Pharm.Sci. 19, 403.

Ciron, C., Lengacher, S., Dusonchet, J., Aebischer, P., Schneider, B.L. (2012). Sustained expression of PGC-1alpha in the rat nigrostriatal system selectively impairs dopaminergic function. Hum Mol Genet. 21, 1861 –1876.

Corbett, A. et al. (2012). Drug repositioning for Alzheimer's disease. Nat rev Drug Discov. 11, 833-846.

Dinamarca, M.C., Arrazola, M., Toledo, E., Cerpa, W.F., Hancke, J., Inestrosa, N.C. (2008). Release of acetylcholinesterase (AChE) from beta-amyloid plaques assemblies improves the spatial memory impairments in APP-transgenic mice. Chem Biol Interact. 175, 142–149.

Drubin, D.G. and Kirschner, M.W. (1986). Tau protein function in living cells. J. Cell Biol.103 , 2739 – 2746.

Eldar-Finkelman, H., Schreyer, S.A., Shinohara, M.M., LeBoeuf, R.C., Krebs, E.G., (1999). Increased glycogen synthase kinase-3 activity in diabetes- and obesity-prone C57BL/6J mice. Diabetes 48, 1662–1666.

Endres, K., Reinhardt, S. (2013). ER-stress in Alzheimer's disease: turning the scale?.Am J Neurodegener Dis. 2, 247-265.

Fagone, P., and Jackowski, S. (2009). Membrane phospholipid synthesis and endoplasmic reticulum function. J. Lipid Res. 50(Suppl.), S311–S316.

Fakhoury, M. (2015). Role of Immunity and Inflammation in the Pathophysiology of Neurodegenerative Diseases. Neurodegener Dis. Epub ahead of print.

Fang, F., Lue. L.F., Yan, S., Xu, H., Luddy, J.S., Chen, D., Walker, D.G., Stern, D.M., Schmidt, A.M., Chen, J.X., Yan, S.S. (2010). RAGE-dependent signaling in microglia contributes to neuroinflammation, Abeta accumulation, and impaired learning/memory in a mouse model of Alzheimer's disease. FASEB J. 24, 1043–1055.

Flaherty, D.B., Soria, J.P., Tomasiewicz, H.G., (2000). Wood JG. Phosphorylation of human tau protein by microtubule-associated kinases: GSK3beta and cdk5 are key participants. J Neurosci Res. 62, 463-72.

Frost, B., Götz, J., Feany, M.B. (2015). Connecting the dots between tau dysfunction and neurodegeneration. Trends Cell Biol. 25, 46-53.

Gao, C., Hölscher, C., Liu, Y., Li, L. (2011). GSK3: a key target for the development of novel treatments for type 2 diabetes mellitus and Alzheimer disease. Rev Neurosci. 23, 1-11.

Gao, C., Liu, Y., Jiang, Y., Ding, J., Li, L. (2014). Geniposide ameliorates learning memory deficits, reduces tau phosphorylation and decreases apoptosis viaGSK3β pathway in streptozotocin-induced alzheimer rat model. Brain Pathol. 24, 261-9.

Galvez, M., Martin-Cordero, C., Ayuso, M.J. (2005). Iridoids as DNA topoisomerase I poisons. J. Enzyme Inhib. Med. Chem. 20, 389.

Gong N, Fan H, Ma AN, Xiao Q, Wang YX (2014) Geniposide and its iridoid analogs

exhibit antinociception by acting at the spinal GLP-1 receptors. Neuropharmacology 84:31-45

Götz, J., Ittner, A., Ittner, L.M. (2012). Tau-targeted treatment strategies in Alzheimer's disease. Br J Pharmacol. 165, 1246-59.

Green, B.D., Gault, V.A., Flatt, P.R., Harriott, P., Greer, B., O'Harte, F.P., (2004). Comparative effects of GLP-1 and GIP on cAMP production,insulin secretion, and in vivo antidiabetic actions following substitution of Ala8/Ala2 with 2-aminobutyric acid. Arch. Biochem. Biophys. 428,136–143.

Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y.C., Zaidi, M.S. (1986). Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem. 261(13), 6084-9.

Godoy, J.A., Rios, J.A., Zolezzi, J.M., Braidy, N., Inestrosa, N.C. (2014). Signaling pathway cross talk in Alzheimer's disease. Cell Commun Signal. 12, 23.

Guo, L.X., Liu, J.H., Xia, Z.N. (2009). Geniposide inhibits CoCl2- induced PC12 cells death via the mitochondrial pathway. Chin Med J (Engl). 122, 2886－92.

Guo, L.X., Xia, Z.N., Gao, X., Yin, F., Liu, J.H. (2012). Glucagonlike peptide 1 receptor plays a critical role in geniposide-regulated insulin secretion in INS-1 cells. Acta Pharmacol Sini. 33, 237-241.

Haan, M.N. (2006). Therapy insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. Nature Clinical Practice. Neurology. 2, 159–166.

Habib, L.K., Lee, M.T., Yang, J. (2010). Inhibitors of catalase-amyloid interactions protect cells from beta-amyloid-induced oxidative stress and toxicity. J Biol Chem. 285, 38933–38943.

Ha, K.S., Kim, K.M., Kwon, Y.G. et al. (2003). Nitric oxide prevents 6-hydroxydopamine- induced apoptosis in PC12 cells through cGMP-dependent PI3 kinase/Akt activation. Faseb J. 17, 1036－47.

Han, H., Yang, L., Xu, Y., Ding, Y., Annie Bligh, S.W., Zhang, T., Wang. Z.T. (2011). Identifi cation of metabolites of geniposide in rat urine using ultra-performance liquid chromatography combined with electrospray ionization quadrupole time-of-fl ight tandem mass spectrometry. Rapid Commun. Mass Spectrom. 25, 3339–3350.

Hartikka, J., Hefti, F. (1988). Comparison of nerve growth factor′ s effects on development of septum，striatum，and nucleus basalis cholinergic neurons in vitro. J Neurosci Res. 21, 352－64

Hashiguchi, M., Hashiguchi, T., (2013). Kinase-kinase interaction and modulation of tau phosphorylation. Int Rev Cell Mol Biol. 300, 121-60.

Heneka, M.T., Kummer, M.P., Latz, E. (2014). Innate immune activation in neurodegenerative disease. Nat Rev Immunol. 14, 463-477.

Hollingworth, P., Harold, D., Jones, L., Owen, M.J., Williams, J. (2011). Alzheimer's disease genetics: current knowledge and future challenges. Int. J. Geriatr. Psychiatry. 26, 793–802.

Hölscher, C. (2010). The role of GLP-1 in neuronal activity and neurodegeneration. Vitam. Horm. 84 , 331 – 354.

Hölscher C (2011) Diabetes as a risk factor for Alzheimer's disease: insulin signalling impairment in the brain as an alternative model of Alzheimer's disease. Biochemical Society transactions 39:891-897.

Hölscher, C. (2014a). Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. Journal of Endocrinology. 221, T31–T41.

Hölscher, C. (2014b). Drugs developed for treatment of diabetes show protective effects in Alzheimer's and Parkinson's diseases. Acta Physiologica Sinica. 66, 497–510.

Hooper, C., Killick, R., Lovestone, S. (2008). The GSK3 hypothesis of Alzheimer's disease. J Neurochem. 104, 1433-9.

Hou, Y.C., Tsai, S.Y., Lai, P.Y., Chen, Y.S., Chao, P.D.L. (2008). Metabolism and pharmacokinetics of genipin and geniposide in rats. Food and Chemical Toxicology. 46, 2764–2769.

Hsu, H.Y., Yang, J.J., Lin, S.Y., Lin, C.C. (1997). Comparisons of geniposidic acid and geniposide on antitumor and radioprotection after sublethal irradiation. Cancer. Lett. 113, 31.

Huang, E.J., Reichard, L.F. (2003). TRK receptors: roles in neuronal signal transduction. Annu. Rev. Biochem. 72, 609–64210.1146.

Huang, L., Wang, C., Naren, G., Aori, G. (2013). Effect of geniposide on LPS-induced activation of TLR4-NF-κB pathway in RAW264.7 macrophage cell line. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 29, 1012-4.

Iqbal, K., Liu, F., Gong, C.X. and Grundke-Iqbal, I. (2010). "Tau in Alzheimer disease and related tauopathies," Current Alzheimer research, vol. 7, no. 8, pp. 656–664.

Ittner,L.M.et al. (2010). Dendritic function of tau mediates amyloid-βtoxicity in Alzheimer's disease mouse models. Cell 142,387-397.

Johnston, H., Boutin, H., Allan, S.M. (2011). Assessing the contribution of inflammation in models of Alzheimer's disease. Biochem. Soc. Trans. 39, 886–890.

Kamenetz, F., Tomita,T., Hsieh,H., Seabrook,G., Borcheit, D., Iwatsubo, T., Sisodia, S., Malinow, R. (2003). APP processing and synaptic function. Neuron.37, 925-937.

Kang, E.B., Kwon, I.S., Koo, J.H., Kim, E.J., Kim, C.H., Lee, J., Yang, C.H., Lee, Y.I., Cho, I.H., Cho, J.Y. (2013). Treadmill exercise represses neuronal cell death and inflammation during Abeta-induced ER stress by regulating unfolded protein response in aged presenilin 2 mutant mice. Apoptosis. 18, 1332-1347.

Koo, H.J., Lim, K.H., Jung, H.J. et al. (2006). Anti-inflammatory evaluation of gardenia extract，geniposide and genipin． J Ethnopharmacol. 103, 496－500.

Koo, H.J., Song, Y.S., Kim, H.J.et al. (2004). Antiinflammatory effects of genipin，an active principle of gardenia． Eur J Pharmacol. 495, 201－8.

Kuo, W., Chou, F., Young, S. et al. (2005). Geniposide activates GSH S- transferase by the induction of GST M1 and GST M2 subunits involving the transcription and phosphorylation of MEK- 1 signaling in rat hepatocytes． Toxicol Appl Pharm. 208, 155－62.

Leclerc, E., Sturchler, E., Vetter, S. (2010). The S100B/RAGE Axis in Alzheimer's

Disease. Cardiovasc Psychiatry Neurol. 2010, 1–11.

Lee, H.P., Zhu, X., Casadesus, G., Castellani, R.J. (2010). Nunomura A, Smith MA, Lee HG, Perry G: Antioxidant approaches for the treatment of Alzheimer's disease. Expert Rev Neurother. 10, 1201 –1208.

Lee, H.Y., Lee, G.H., Lee, M.R., Kim, H.K., Kim, N.Y., Kim, S.H., Lee, Y.C., Kim, H.R., Chae, H.J. (2013). Eucommia ulmoides Oliver extract, aucubin, and geniposide enhance lysosomal activity to regulate ER stress and hepatic lipid accumulation. PLoS One. 8, e81349.

Liu, H.T., He, J.L., Li, W.M., Yang, Z., Wang, Y.X., Yin, J., Du, Y.G., Yu, C. (2010). Geniposide inhibits interleukin-6 and interleukin-8 production in lipopolysaccharide-induced human umbilical vein endothelial cells by blocking p38and ERK1/2 signaling pathways. Inflammation Res. 59, 451.

Liu, J.H., Yin, F., Guo, L.X., Deng, X.H., and Hu, Y.H. (2009). Neuroprotection of geniposide against hydrogen peroxide induced PC12 cells injury: involvement of PI3 kinase signal pathway. Acta Pharmacol. Sin. 30 , 159– 165.

Liu, J., Yin, F., Xiao, H., Guo, L., Gao, X., (2012). Glucagon-like peptide 1 receptor plays an essential role in geniposide attenuating lipotoxicity-induced β-cell apoptosis. Toxicol In Vitro. 26, 1093-1097.

Liu, J., Yin, F., Zheng, X. et al. (2007). Geniposide, a novel agonist for GLP-1 receptor, prevents PC12 cells from oxidative damage via MAP kinase pathway. Neurochem Int. 51, 361－9.

Liu, J., Zheng, X., Yin, F. et al. (2006). Neurotrophic property of geniposide for inducing the neuronal differentiation of PC12 cells [J]. Int J Dev Neurosci. 24, 419－24.

Li, L. and Holscher, C. (2007). Common pathological processes in Alzheimer disease and type 2 diabetes: a review. Brain Res Rev. 56, 384–402.

Li, Y., Duffy, K.B., Ottinger, M.A., Ray, B., Bailey, J.A., Holloway,H.W., Tweedie, D., Perry, T., Mattson, M.P., Kapogiannis, D.,et al. (2010). GLP-1 receptor stimulation reduces amyloid- β peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer ’ s disease. J. Alzheimers Dis. 19 , 1205 – 1219.

Llorens-Mart í n, M., Jurado, J., Hern á ndez, F., Avila, J.(2014) GSK-3 β , a pivotal kinase in Alzheimer disease. Front Mol Neurosci. 7, 46.

Lue, L.F., Walker, D.G., Brachova, L., Beach, T.G., Rogers, J., Schmidt, A.M., Stem, D.M., Yan, S.D. (2001). Involvement of microglial receptor for advanced glycation end products (RAGE) in Alzheimer's disease: identification of a cellular activation mechanism. Exp Neurol. 171, 29–45.

Lv, C., Liu, X., Liu, H., Chen, T., Zhang, W. (2014). Geniposide attenuates mitochondrial dysfunction and memory deficits in APP/PS1 transgenic mice. Curr Alzheimer Res. 11, 580-7.

Lv, C., Wang, L., Liu, X., Yan, S., Yan, SS., Wang, Y., Zhang, W. (2015). Multi-faced neuroprotective effects of geniposide depending on the RAGE-mediated signaling in an Alzheimer mouse model. Neuropharmacology. 89,175-84.

Mathis, C.A.， Lopresti, B.J.， Klunk, W.E． (2007). Impact of amyloid imaging on drug development in Alzheimer′ s disease. Nucl Med Biol， 2007，34, 809－

22.

Ma, T., Huang, C., Zong, G., Zha, D., Meng, X., Li, J., Tang, W. (2011). Hepatoprotective effects of geniposide in a rat model of nonalcoholic steatohepatitis. J. Pharm. Pharmacol.

Matsumi, Y., Kenzo, C., Keiko, S. (2008). Neuro2a cell death induced by 6-hydroxydopamine is attenutated by genipin. J Health Sci. 54, 638－44.

Ma, T., Tan, M., Yu, J., Tan, L. (2014). Resveratrol as a Therapeutic Agent for Alzheimer's Disease .Biomed Res Int. 2014,350516.

McClean, P.L., Parthsarathy, V., Faivre, E., and H ö lscher, C. (2011). The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer ' s disease. J. Neurosci. 31, 6587 – 6594.

Morris GP, Clark IA, Vissel B (2014) Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta neuropathologica communications 2:135.

McGeer, P.L., Itagaki, S., Boyes, B.E., McGeer, E.G. (1988). Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains.Neurology. 38, 1285–1291.

Meares, G.P., Mines, M.A., Beurel, E., Eom, T.Y., Song, L.,Zmijewska,A.A., and Jope, R.S. (2011). Glycogen synthase kinse-3 regulates endoplasmic reticulum (ER) stress-induced CHOP expression in neuronal cells. Exp. Cell Res. 317, 1621–1628.

Meda, L., Cassatella, M.A., Szendrei, G.I., Otvos Jr, L., Baron, P. Villalba, M. et al. (1995). Activation of microglial cells by beta-amyloid protein and interferon-gamma. Nature. 374 (6523) pp, 647–650

Medina, M., Avila, J. (2014). New perspectives on the role of tau in Alzheimer's disease. Implications for therapy. Biochem Pharmacol. 88, 540-7.

Murakami, T., Hino, S.I., Saito, A., Imaizumi, K., (2007). Endoplasmic reticulum stress response in dendrites of cultured primary neurons. Neuroscience. 146, 1-8.

Nelson, T.J., Alkon, D.I., (2005). Insulin and cholesterol pathways in neuronal function, memory and neurodegeneration. Biochem Soc Trans. 33,1033–6.

Parihar, M.S., Brewer, G.J. (2010). Amyloid-β as a modulator of synaptic plasticity. J.Alzheimers Dis .22,741-763.

Pan L, Zhou J, Zhu H, Wang W, Zhang M, Tian X, Lu J, Zeng M (2014) Study on integrated pharmacokinetics of gardenia acid and geniposide: time-antioxidant efficacy after oral administration of Huanglian-Zhizi couplet medicine from Huang-Lian-Jie-Du-Tang in MCAO rats. The American journal of Chinese medicine 42:393-407

Patapoutian, A., Reichardt, L.F. (2001). Trk receptors: mediators of neurotrophin action [J]. Curr Opin Neurobiol. 11, 272－80.

Perry, T., Lahiri, D.K., Chen, D. et al. (2002). A novel neurotrophic property of glucagon-likepeptide1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. J Pharmacol Exp Ther. 300, 958－66.

Perry, T., Lahiri, D.K., Sambamurti, K., Chen, D., Mattson, M.P., Egan, J.M., et al., (2003). Glucagon-like peptide-1 decreases endogenous amyloid beta peptide

(Abeta) levels and protects hippocampal neurons from death induced by Abeta and iron. J. Neurosci. Res. 72, 603–612.

Perry T, Greig NH (2005) Enhancing central nervous system endogenous GLP-1 receptor pathways for intervention in Alzheimer's disease. Current Alzheimer research 2:377-385.

Piaceri, I., Rinnoci, V., Bagnoli, S., Failli, Y., Sorbi, S. (2012). Mitochondria and Alzheimer's disease. J Neurol Sci. 322, 31 –34.

Pimplikar, S.W. (2009). Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 41, 1261-1268.

Raff, M.C., Whitmore, A.V., Finn, J.T. (2002). Axonal self-destruction and neurodegeneration. Science. 296, 868-871.

Rakez, K., Cristian, A.L.R. (2013). Molecular Mechanisms of Amyloid Oligomers Toxicity. J.Alzheimer's Dis. 33, s67-s78.

Ristow, M. (2004). Neurodegenerative disorders associated with diabetes mellitus. Journal of Molecular Medicine. 82, 510–529.

Rubio-Perez, J.M., Morillas-Ruiz, J.M. (2012). A review: inflammatory process in Alzheimer's disease, role of cytokines. ScientificWorldJournal.2012, 756357.

Sabbagh, M.N., Galasko, D., Koo, E., Thal, L.J. (2000). Amyloid-beta and treatment opportunities for Alzheimer's disease. J Alzheimers Dis. 2, 231-59.

Salminen, A., et al. (2010). Endoplasmic reticulum stress in age-related macular degeneration: trigger for neovascularization. Mol. Med. 16, 535e542.

Sammels, E., Parys, J.B., Missiaen, L., De Smedt, H., Bultynck, G. (2010). Intracellular Ca2+ storage in health and disease: a dynamic equilibrium.Cell Calcium 47, 297–314.

Selkoe, D.J. (2008). Soluble oligomers of the amyloid-β protein impair synaptic plasticity and behavior. Behav. Brain.Res.192.106-113.

Shao, S., Liu, Z., Yang, Y., Zhang, M., Yu, X. (2010). SREBP-1 c,Pdx-1 , and GLP-1 R involved in palmitate-EPA regulated glucose-stimulated insulin secretion in INS-1 cells. J Cell Biochem. 111, 634-642.

Sharma M, Jalewa J, Holscher C (2013) Neuroprotective and anti-apoptotic effects of Liraglutide on SH-SY5Y cells exposed to Methylglyoxal stress. Journal of neurochemistry 128:459-471.

Song, X., Zhang, W., Wang, T., Jiang, H., Zhang, Z., Fu, Y., Yang, Z., Cao, Y., Zhang, N. (2014). Geniposide plays an anti-inflammatory role via regulating TLR4 and downstream signaling pathways in lipopolysaccharide-induced mastitis in mice. Inflammation. 37, 1588-98.

Stalder, M., Phinney, A., Probst, A., Sommer, B., Staufenbiel, M.,and Jucker, M. (1999). Association of microglia with amyloid plaques in brains of APP23 transgenic mice. Am. J. Pathol. 154 ,1673 – 1684.

Sudduth, T.L., Schmitt, F.A., Nelson, P.T., Wilcock, D.M. (2013). Neuroinflammatory phenotype in early Alzheimer's disease. Neurobiol. Aging. 34, 1051–1059.

Sun, Y., Feng, F., Yu, X. (2012). Pharmacokinetics of geniposide in Zhi-Zi-Hou-Pu decoction and in different combinations of its constituent herbs. Phytother Res. 26, 67–72.

Tanaka, M., Yamazaki, M., Chiba, K. (2009). Neuroprotective action of genipin on tunicamycin-induced cytotoxicity in neuro2a cells． Biol Pharm Bull. 32, 1220－3.

Tan, CC., Yu, JT., Wang, HF., Tan, MS., Meng, XF., Wang, C., Jiang, T., Zhu, XC., Tan, L. (2014). Efficacy and safety of donepezil, galantamine, rivastigmine, and memantine for the treatment of Alzheimer's disease: a systematic review and meta-analysis. J Alzheimers Dis. 41, 615-31.

Terwel, D., Dewachter, I., Van Leuven, F. (2002). Axonal transport, tau protein, and neurodegeneration in Alzheimer's disease. Neuromolecular Med. 2, 151-65.

Thinakaran, G., Koo, E.H., (2008). Amyloid precursor protein trafficking,processing,and function. J.Biol.chem. 283, 29615-29619.

Torres, M., Matamala, J.M., Duran-Aniotz, C., Cornejo, V.H., Foley. A., Hetz, C. (2014). ER stress signaling and neurodegeneration: At the intersection between Alzheimer's disease and Prion-related disorders. Virus Res. Epub ahead of print.

Ueno, K., Takeda, Y., Iwasaki, Y., Yoshizaki, F. (2001). Simultaneous estimation of geniposide and genipin in mouse plasma using high-performance liquid chromatography. Anal. Sci. 17, 1237–1239.

Wang, F., Cao, J., Hao, J., Liu, K. (2014). Pharmacokinetics, bioavailability and tissue distribution of geniposide following intravenous and peroral administration to rats. Biopharm Drug Dispos. 35, 97-103.

Wang, J., Hou, J., Zhang, P., Li, D., Zhang, C., Liu, J. (2012). Geniposide reduces inflammatory responses of oxygen-glucose deprived rat microglial cells via inhibition of the TLR4 signaling pathway. Neurochem Res. 37, 2235-48.

Wang, J., Li, P.T., Du, H., Hou, J.C., Li, W.H., Pan, Y.S., Chen, H.C. (2012). Tong Luo Jiu Nao injection, a traditional Chinese medicinal preparation, inhibits MIP-1 expression in brain microvascular endothelial cells injured by oxygen-glucose deprivation. J Ethnopharmacol 141,151–157.

Wang, S.W., Lai, C.Y., Wang, C.J. (1992). Inhibitory effect of geniposide on aflatoxin B1-induced DNA repair synthesis in primary cultured rat hepatocytes. Cancer Lett. 65, 133–137.

Wang, X.H., Li, L., H ö lscher, C., Pan, Y.F., Chen, X.R., and Qi,J.S. (2010). Val8-glucagon-like peptide-1 protects against A β 1-40-induced impairment of hippocampal late-phase long-term potentiation and spatial learning in rats. Neuroscience 170 ,1239 – 1248.

Werner, JG., Altaf, SD. (2015). Pharmacotherapy of Alzheimer’s disease: current and future trends. Expert Rev. Neurother. 15, 3-5.

Wright, J.W., Kawas, L.H., Harding, J.W. (2014). The development of small molecule angiotensin IV analogs to treat Alzheimer's and Parkinson's diseases. Prog Neurobiol. 125C, 26-46.

Wu, R.G., Qiu, L., Zhang, Y., Zhang, Z.J., Luo, Y.J., Wang, Y.Y. (2009). Microarray and proteomic characterization ofmolecular mechanism ofgeniposide in ischemiareperfusion and computer-automated estimation of the possible drug target network, Neurosci. Res. 65, S122.

Yamamoto, M., Miura, N., Ohtake, N. (2000). Genipin，a metabolite derived from the

herbal medicine Inchin-ko-to，and suppression of Fas-induced lethal liver apoptosis in mice． Gastroenterology. 118, 380－9．

Yamazaki, M., Chiba, K., Mohri, T. (1996). Neuritogenic effect of natural iridoid compounds on PC12h cells and its possible relation to signaling protein kinases [J]. Biol Pharm Bull. 19, 791－5．

Yamazaki, M., Chiba, K., Mohri, T. (2001). Activation of the mitogenactivated protein kinase cascade through nitric oxide synthesis as a mechanism of neuritogenic effect of genipin in PC12h cells. J Neurochem. 79, 45－54．

Yamazaki, M., Chiba, K., Mohri, T. et al. (2004). Cyclic GMP-dependent neurite outgrowth by genipin and nerve growth factor in PC12h cells. Eur JPharmacol. 488, 35－43.

Yamazaki, M., Chiba, K., Mohri, T. (2006). Differences in neuritogenic response to nitric oxide in PC12 and PC12h cells. Neurosci Lett. 393 , 222－5.

Yamazaki, M., Chiba, K., Yoshikawa, C. (2009). Genipin suppresses A23187-induced cytotoxicity in neuro2a cells． Biol Pharm Bull. 32, 1043－6.

Yamazaki, M., Sakura, N., Chiba, K. (2001). Prevention of the neurotoxicity of the amyloid beta protein by genipin． Biol Pharm Bul. 24, 1454－5．

Yan, S.F., Du, Yan, S., Ramasamy, R., Schmidt, A.M. (2009). Tempering the wrath of RAGE: An emerging therapeutic strategy against diabetic complications, neurodegeneration, and inflammation. Annals of medicine. 2009,1–15.

Yan, S.S., Chen, D., Yan, S., Guo, L., Du, H., Chen, J.X. (2012). RAGE is a key cellular target for Abeta-induced perturbation in Alzheimer's disease. Front Biosci (Schol Ed). 4, 240–250.

Ye, G., Zhu, H.Y., Zhao, H.L., Xu, B., Huang, C.G., (2006). HPLC method for the determination and pharmacokinetic studies on geniposide in rat serum after oral administration of traditional Chinese medicinal preparation Yin-Zhi-Ku decoction. Biomed. Chromatogr. 20, 743–747.

Yin, F., Liu, J.H., Zheng, X. et al. (2010). Geniposide induces the expression of heme oxygenase- 1 via PI3K/Nrf2- signaling to enhance the antioxidant capacity in primary hippocampal neurons． Biol Pharm Bull. 33, 1841－6．

Yin, F., Liu, J.H., Zheng, X. X. et al. (2010). GLP- 1 receptor plays a critical role in geniposide- induced expression of heme oxygenase- 1 in PC12 cells. Acta Pharmacol Sin. 31, 540－5.

Yin, F., Zhang, Y., Guo, L., Kong, S., Liu, J. (2012). Geniposide regulates insulin-degrading enzyme expression to inhibit the cytotoxicity of $A\beta_{1-42}$ in cortical neurons. CNS Neurol Disord Drug Targets. 11, 1045-51.

Yu, B., Ruan, M., Cui, X.B., Guo, J.M., Xu, L., Dong, X.P., (2013). Effect of borneol on the pharmacokinetics of geniposide in cortex, hippocampus, hypothalamus and striatum of conscious rat by simultaneous mimicrodialysis coupled with UPLC-MS. J Pharm Biomed Anal. 77,128-32.

Yu, Y., Xie, Z.L., Gao, H. (2009). Bioactive iridoid glucosides from the fruit of Gardenia jasminoides. J Nat Prod. 72, 1459－64．

Zheng, X., Yang, D., Liu, X. et al. (2010). Identification of a new anti-LPS agent，geniposide from Gardenia jasminoides Ellis，and its ability of direct binding and

neutralization of lipopolysaccharide in vitro and in vivo. Int Immunopharmacol. 10, 1209－19.

Zhu, J., Gao, X., Xie, W.L. et al. (2005). Effect of geniposide on serum IL-1 beta and TNF-alpha of rheumatoid arthritis rats. China J Chin Mat Med. 30, 708－11.

Tables

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

Parameters	Geniposide(40.65 mg/kg) Zhi-Zi-Hou-Pu decoction	Geniposide(100mg/kg)
Tmax(min)	0.79±0.19	0.5±0.03
Cmax(ug/ml)	1.29±0.16	1.40±0.24
T1/2(h)	2.67±0.56	3.55±0.69
AUC(0-∞) (h·ug/ml)	5.07±1.07	6.76±1.23

(Yu et al., 2013)

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

parameters	Cortex	Hippocampus	Hypothalamus	Striatum
Tmax(min)	24.00±8.94	20.00±0.00	20.00±0.00	20.00±0.00
Cmax(ug/ml)	565.80±234.21	134.87±49.00	133.13±97.76	150.46±63.02
T1/2(h)	1.84±0.80	2.62±2.03	1.69±1.34	2.12±0.75
AUC(0-∞) (h·ug/ml)	796.67±240.00	400±240.00	298.33±96.17	441.67±109.17
MRT(0-∞) (h)	2.04±0.77	3.85±2.79	2.69±1.43	3.25±0.85

(Yu et al., 2013)

Figures:

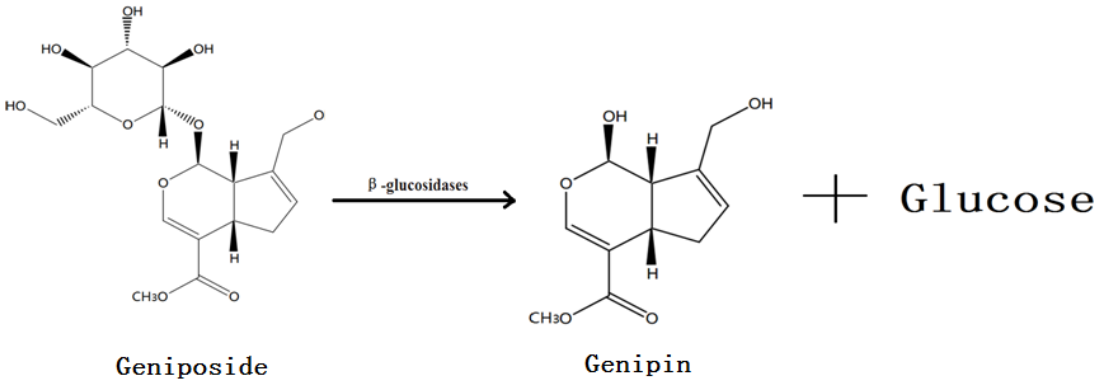

Fig. 1. Mechanism of transforming geniposide into genipin

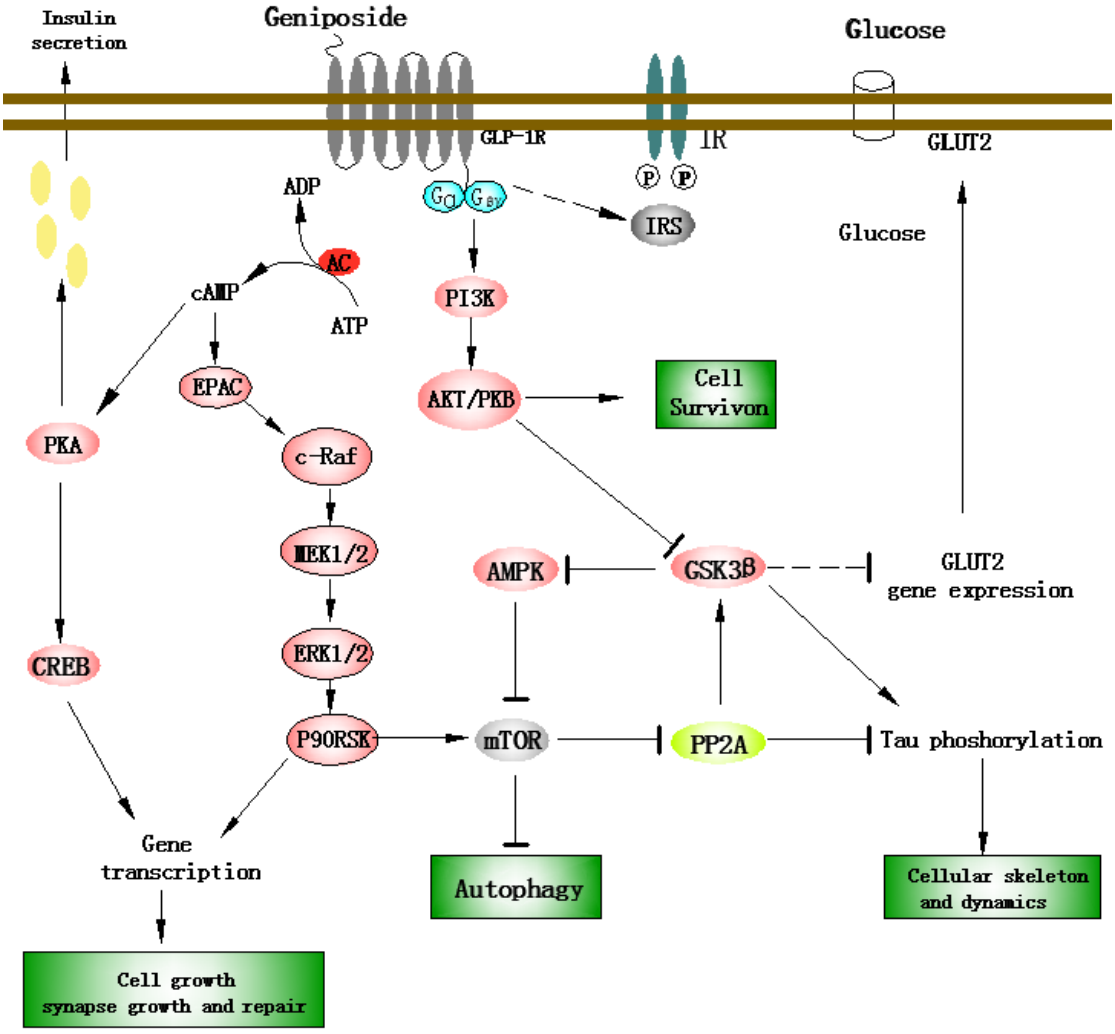

Figure 2: Overview of the main pathways induced by geniposide in neurons Geniposide activated the GLP-1 receptor in a manner similar to GLP-1. The GLP-1 receptor is a member of a different class of receptors compared with insulin receptor (IR). Activation of the GLP-1 receptor activates an adenylyl cyclase and increases cAMP levels (Green et al., 2004), which stimulates protein kinase A (PKA) and enhances the transcription of insulin receptor substrate 2 (IRS2) (Broca et al., 2009). By this pathway it can link with the signaling pathway of IR. Phosphorylation of PKA and other downstream kinases are related to insulin secretion and growth factor signaling. An increase of PI3K levels via the G protein activation can activate following pathways: (1) MAPK. This pathway activates gene expression, which controls 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 stimulation of PI3K binding to IRS and G protein, activation of PI3K and protein kinase B (Akt/PKB), which suppresses the induction of apoptosis and thereby protects neurons (Liu et al., 2012). (3) Activation of Glycogen Synthase Kinase (GSK3) to modify the cellular skeleton and dynamics by mediating the phosphorylation levels of tau protein; modulating cleavage of amyloid-beta

protein precursor (APP) and improve learning and memory formation (Eldar-Finkelman et al., 1999; Gao et al., 2011; Gao et al., 2014). As well, AMPK inhibits mTOR 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, 2014).