Ginkgolides and bilobalide for treatment of Alzheimer's disease and COVID-19: potential mechanisms of action

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Abstract. – Alzheimer's disease (AD) is an irreversible degenerative illness of the central nervous system with characteristic histological alterations, known as amyloid plaques and neurofibrillary tangles (NFT). Aggregation of plaques and tangles in the brain induces neurotoxicity and synaptic dysfunction, eventually contributing to neuronal cell death and neurodegeneration. Recent studies have revealed that COVID-19 has a great impact on the development of AD, directly or indirectly, by facilitating the accumulation of amyloid plaques, causing altered functional brain integrity or increasing the phosphorylation rate of tau protein. As two important bioactive components of *Ginkgo biloba* extract (GbE), ginkgolides and bilobalide (BB) have been reported to show neuroprotective effects in AD via multiple mechanisms such as anti-excitotoxicity, anti-inflammatory and anti-oxidative activities. Intriguingly, ginkgolides and BB also seem to demonstrate antiviral properties against COVID-19 by inhibiting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) main protease. Herein, we review studies on the neuroprotective and antiviral mechanisms of ginkgolides and bilobalide, as well as their therapeutic potential against AD and COVID-19.

Key Words:
   Ginkgo biloba extract, Ginkgolides, Bilobalide, Alzheimer's disease, COVID-19, Pathogenesis.

Introduction

Alzheimer's disease (AD) is an irreversible degenerative illness of the central nervous system (CNS) characterised mainly by histological alterations known as amyloid plaques and neurofibrillary tangles (NFT), which was first reported by Dr. Alois Alzheimer in 1906[1]. According to the World Alzheimer Report 2015, over 46 million individuals worldwide were affected by AD in 2015, and this number was expected to increase to 131.5 million by 2050[2]. Currently, both acetylcholinesterase inhibitors (AChEIs) and N-methyl-D-aspartate receptor (NMDAR) antagonists are recommended for the treatment of AD. However, these drugs only provide temporary relief of symptoms; in essence, they neither slow down the development of dementia, nor show good results with some side effects after long-term use. Although an assortment of novel agents, including aducanumab and GV-971 (a marine algae-derived oral oligosaccharide), have been approved for clinical use, they have not been completely applied in clinical practice because of controversial therapeutic effects[3-6]. Under the above-mentioned circumstances, botanical preparations with multi-target treatment and safety have become feasible as new treatment approaches for AD. Various *in vivo* and *in vitro* studies[7-10] have recommended the therapeutic potential of resveratrol, Rhodiola sachalinensis, curcumin, and natural polyphenols for the prevention and treatment of AD.

Apart from these botanicals, *Ginkgo biloba* extract (GbE) has gained increasing popularity and is generally utilized for the treatment of CNS and cardiovascular disorders with multiple biological characteristics through various mechanisms, such as anti-oxidant, free radical-scavenging, platelet accumulation inhibiting and anti-inflammatory activities[11-15]. In addition, there is growing evidence demonstrating the efficacy of GbE in the treatment of dementia[16-18]. Consequently, the Asian expert consensus in 2021 recommended GbE as a treatment option for dementia and mild cognitive impairment without cerebrovascular disease, particularly for those unresponsive to AChEIs and NMDAR antagonists[19]. In the 1970s, Dr. Willmar Schwabe Pharmaceuticals[20,21] (Willmar-Schwabe-Street 4, Karlsruhe, Germany) extracted EGb 761, a high-level concentration and stable extract, originating from *Ginkgo bilo-*

ba leaves by means of ethanol reflux extraction. Generally, GbE is composed of 22-27% ginkgo flavone (mainly comprising quercetin, kaempferol, and isorhamnetin), 5-7% terpene lactone (including ginkgolides and bilobalide), and less than 5 ppm of ginkgolic acid[22-25]. Increasing evidence has confirmed that ginkgolides and bilobalides have extensive neuroprotective properties for the treatment of AD to some extent[26,27].

Besides AD, the COVID-19 pandemic, which has caused global morbidity and mortality, has been reported to increase the risk of mortality by five times in the elderly people compared with the general population[28]. Patients with AD are seven times more at risk of being infected with COVID-19, with the mortality rate increased by two-folds[29]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen responsible for COVID-19, can cause neurological signs and symptoms (i.e., headache, impaired consciousness, delirium, hypogeusia/ageusia, encephalitis, seizures, strokes, cognitive and memory deficits) by directly invading the CNS, which results in subsequent interaction between SARS-CoV-2 spike protein and angiotensin-converting enzyme 2 (ACE2)[30-32]. Post-mortem examinations have demonstrated the presence of both SARS-CoV-2 protein and RNA in the brain tissue of COVID-19 patients[33]. Despite the lack of evidence regarding its anti-COVID-19 properties, ginkgolic acid has been reported to effectively suppress human immunodeficiency virus-1 (HIV-1) protease activity in a cell-free system and HIV infection in human peripheral blood mononuclear cells (PBMCs) with negligible cytotoxicity[34], indicating the therapeutic potential of ginkgolides against COVID-19 infection. This narrative review summarises the mechanisms of action underlying neuroprotection *via* ginkgolides and bi-

lobalide (BB) against AD and, to a lesser extent, the antiviral properties against COVID-19.

Bioactive Mechanisms and Properties of Ginkgolides and Bilobalide

Ginkgolides and BB are terpene lactones and two important bioactive components of GbE[35]. For decades, ten kinds of diterpenoids, namely ginkgolides A, B, C, J, K, L, M, N, P, and Q, have been isolated from *Ginkgo biloba* leaves[36] (Figure 1A). The aforementioned components demonstrated multiple biological activities, including anti-apoptosis, anticoagulant, anti-inflammatory, and antioxidant effects[37] (Table I). As another constituent of GbE, BB is a naturally occurring sesquiterpene trilactone that has neuroprotective, antioxidative, anti-inflammatory, anti-ischaemic, and cardiovascular protective activities[26] (Figure 1B).

AD Pathogenesis and COVID-19

Although the pathogenesis of AD remains unclear, it has been confirmed that amyloid β (Aβ) and NFTs play major roles in the development of this disease[38]. The characteristic histopathological features of AD are the extracellular aggregation of Aβ plaques and intracellular deposits of neurofibrillary tangles consisting of hyperphosphorylated microtubule-associated protein. Under physiological circumstances, Aβ exists as a proteolytic fragment of larger β-amyloid precursor protein (APP) and is produced by the sequential cleavage of the β-secretase and γ-secretase complex[39]. Under pathological circumstances, the abnormal sequential cleavage of APP by β-secretase and γ-secretase occurs, resulting in the overpro-

Table I. Functions of ginkgolides; based on S.H. Omar "Ginkgolides and Neuroprotective Effects"[37].

Function	Ginkgolide	Effect
Anti-inflammatory	A, B, C, J	IL-5 and IL-13 inhibition; mitogen-activated protein kinase (MAPK) inhibition; toll-like receptor (TLR) inhibition; Inhibitor of arachidonic acid inflammatory pathway
Anti-coagulant	B	PAF (platelet-activating factor) inhibition
Anti-oxidative	A, B,	Nuclear factor kappa B (NF-kB) inhibition
Anti-apoptotic	A, C, J, M, K	TNF receptor inhibition
Anti-oxidative	B, C, J, M	Free radical scavengers

IL: interleukin; TNF: tumor necrosis factor.

Figure 1. Structure of ginkgolides (**A**) and bilobalide (**B**).

duction of Aβ peptide and the release of extracellular Aβ40/Aβ42, which then aggregates to form plaques in the brain. This concentration of Aβ triggers the activation of kinases, which leads to hyperphosphorylation of microtubule-associated τ protein and NFTs formation thereafter. The aggregation of plaques and tangles, which is followed by the recruitment of microglia around plaques, further promotes microglial activation and local inflammatory reactions and induces neurotoxicity and synaptic dysfunction, ultimately contributing to neuronal cell death and neurodegeneration[38,40] (Figure 2).

The possible mechanisms underlying neurological deficits in COVID-19, such as cognitive impairment that may eventually result in AD, include COVID-19-induced inflammation, SARS-CoV-2 invasion of the CNS, long-term hospitalisation and delirium, and post-COVID-19 syndrome[41]. Several pathological mechanisms have been proposed to explain the impact of COVID-19 on the development of AD. First, virus-induced systemic inflammation leads to the disruption of the blood-brain barrier and causes neural and glial cell damage. Essentially, pro-inflammatory cytokines (e.g., interleukin [IL]-1β, IL-6, IL-12, and tumour necrosis factor [TNF]-α) alter the capacity of microglial cells to phagocytose Aβ, prompting the accumulation of amyloid plaques[42]. Second, hypoxic alterations, as described in COVID-19 patients, can cause altered functional brain integrity, especially in the hippocampus[43,44], and cerebral ischaemia can increase the phosphorylation rate of tau protein, potentially resulting in NFTs formation[45]. Third, SARS-CoV-2 neuroinvasion might promote Aβ generation partly due to the immune response and the Aβ cascade leads to Aβ deposition in the brain[46]. Nevertheless, further evidence is required to prove these hypotheses.
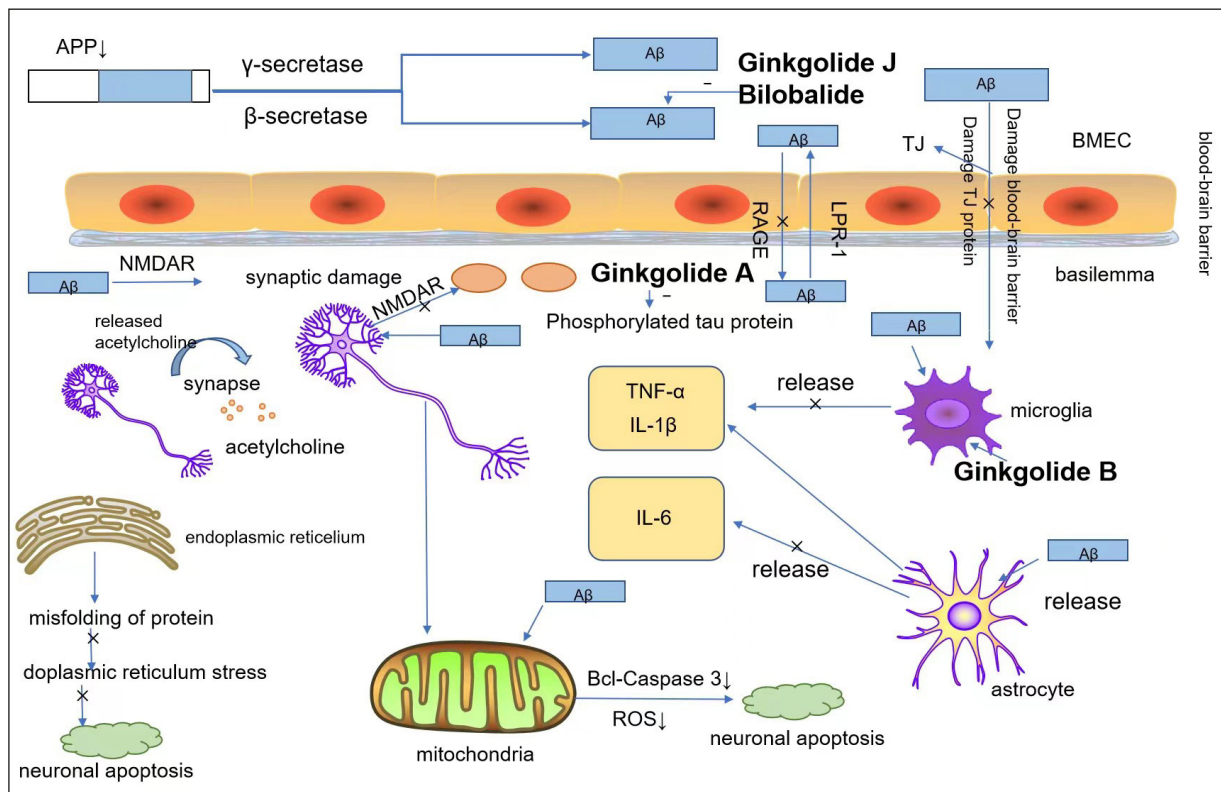
Figure 2. Pathogenesis of Alzheimer's disease and COVID-19 as well as neuroprotective mechanisms of ginkgolides and bilobalide. Aβ: amyloid β; APP: β-amyloid precursor protein; BMEC: brain microvessel endothelial cell; IL: interleukin; LPR-1: low density lipoprotein receptor-related protein 1; NFT: neurofibrillary tangles; NMDAR: N-methyl-D-aspartate receptor; ROS: reactive oxygen species; TNF: tumor necrosis factor; TJ: tight junction.

Therapeutic Potential of Various Ginkgolides Components in Treatment of AD by Neuroprotective Mechanisms

Ginkgolide A (GA, BN52020) is an effective antagonist of phospholipid-derived platelet-activating factor (PAF), which is involved in the immune response to infection and neuronal damage caused by ischaemic and cytotoxic injury[47-49]. In a model organism of Caenorhabditis elegans, Wu et al[50] found that EGb 761 and GA attenuated 5-HT hypersensitivity and chemotactic activity and alleviated Aβ-triggered pathological behaviour such as paralysis in the model organism. Using a screening platform, GA was demonstrated to reduce the Aβ-induced abnormal depolarization of primary cortical neurons in the mouse brain. After administration of the receptor agonist, GA was shown to suppress the α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid receptor and NMDAR, and further prevent Aβ-induced increase in the phosphorylation of c-Jun N-terminal kinase in neurons. When wild-type and AD animal model mice with or without GA treatment were compared, other parameters, such as body weight, glutamic oxaloacetate transaminase, glutamic-pyruvic transaminase, liver histology, and kidney histology, were found to be similar. Notably, the pure compound was able to improve memory in wild type mice[51]. Based on an *in vitro* experiment[52], it was confirmed that GA improved the cell viability of N2a cell lines and inhibited the phosphorylation level of Tau in cell lysates but restrained the phosphorylation of GSK3β at Ser9. Additionally, treatment with GA promoted the intracellular phosphorylation of PI3K and Akt, suggesting that the activation of the PI3K-Akt signalling pathway may be the mechanism of action of GA to prevent the intracellular accumulation of P-tau induced by okara acid, and hence protect the cells from toxicity caused by Tau hyperphosphorylation. Taken together, these findings indicate the therapeutic potential of GA against AD and related tauopathies.

As one of the key components of EGb 761, ginkgolide B (GB, BN52021) is the most effective PAF antagonist. In an *in vitro* experiment[53] using cortical or hippocampal neurons, pre-treatment of cortical or hippocampal neurons with GA or GB protected against Aβ1-42-induced loss of synaptophysin. PAF had Aβ1-42-mimicking effects that caused a dose-dependent decrease in synaptophysin expression in neurons; however, this effect was largely abrogated by pretreatment with GB. A study[54] on the effect of GB on the release of endogenous glutamate from rat hippocampal nerve terminals (synaptosomes) revealed that GB promoted the Ca2+ dependent release of glutamate induced by 4-aminopyridine in a concentration-dependent manner, which could be attributed to the enhancement of presynaptic voltage-dependent Ca2+ influx. Consistent with this, GB-mediated promotion of glutamate release was significantly inhibited in synaptosomes pre-treated with the calcium channel blocker ω-conotoxin MVIIC. In addition, the enhancement action of GB was completely eliminated by a protein kinase A inhibitor. These results suggest that GB could enhance the increase in protein kinase A activation and then facilitate Ca2+ entry through voltage-dependent N-type and P/Q-type Ca2+ channels, leading to increased glutamate release from rat hippocampal nerve terminals. Interestingly, GB also promotes the release of glutamic acid elicited by the Ca2+ ionophore (ionomycin), implicating the direct effects of GB on the secretory apparatus downstream of Ca2+ entry.

In an *in vitro* experiment[55] using two cell models of SH-SY5Y neuroblastoma cells and primary cortical neurons, treatment with GA or GB protected against synthetic miniprion (sPrP106) or Aβ1-42-induced apoptosis in cortical neurons by reducing either caspase-3 expression or microglial killing of neurons in response to Aβ1-42 or sPrP106. Ginkgolide-treated cells were also resistant to arachidonic acid or PAF and displayed reduced production of prostaglandin E2 in response to Aβ1-42 or sPrP106. Additionally, GB pre-treatment substantially decreased the levels of reactive oxygen species (ROS)/ reactive nitrogen species (RNS) and increased the levels of mitochondrial APE1 in human neuroblastoma IMR-32 and SH-SY5Y cells in response to Aβ25-35 peptide. This was because of the regulatory effects of GB on oxidative phosphorylation (OXPHOS; the activities of complexes I, III, and IV) that was caused by upregulating the activities of complexes I and IV, rendering neuronal cells resistant to oxidative stress

induced by Aβ25-35[56]. In human neuroblastoma SH-SY5Y cells pre-treated with Aβ25-35 for 24 h, treatment with GB demonstrated neuroprotective potential by reducing the production of ROS and RNS, lipid peroxidation, and protein carbonyl content, as well as restoring the antioxidant activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzymes[57]. Interestingly, Wang et al[58] recently reported that GB alleviated Aβ1-42-induced apoptosis and reversed Aβ1-42-induced oxidative stress by decreasing the levels of SOD, GSH-Px, and ATP as well as increasing the levels of malondialdehyde (MDA) and ROS in astrocytes. In addition, GB decreased the expression levels of endoplasmic reticulum stress (ERS) protein and apoptosis protein CHOP but increased the mRNA and protein expression of AMPK/peroxisome proliferator-activated receptor γ coactivator 1α/peroxisome proliferator-activated receptor α and nuclear respiratory factor 2/heme oxygenase 1/ NAD(P)H dehydrogenase (quinone 1), as well as the protein expression of β-secretase 1. It is noteworthy that GB protected against Aβ1-42-induced apoptosis by inhibiting ERS, oxidative stress, and energy metabolism dysfunction *via* the activation of AMPK signalling pathways. The multiple beneficial mechanisms of action make GB a potential treatment option for AD.

Ginkgolide J (GJ) possesses anti-inflammatory property[59]. To date, few studies have been published concerning the treatment of AD using GJ. In an *in vitro* study by Vitolo et al[60], a new *Ginkgo biloba* extract, P8A, was shown to prevent Aβ1-42-induced suppression of long-term potentiation (indicative of synaptic dysfunction) in the CA1 region of mouse hippocampal slices, mainly attributed to GJ, which fully replicates the effect of the extract. Moreover, GJ inhibited Aβ1-42-induced cell death in rat fetal hippocampal neurons[60]. These findings strongly suggest that GJ may protect against synapse damage and cell loss during the early stages of AD. Nevertheless, more evidence is needed to clarify this issue.

Therapeutic Potential of Bilobalide in Treatment of AD by Neuroprotective Mechanisms

As a trilactone terpene, BB is structurally similar to ginkgolide. It is not a direct PAF antagonist but is capable of exerting synergistic effects by reducing the levels of PAF receptors along with ginkgolides-like PAF antagonist[61]. In a mouse model of AD, BB and

quercetin remarkably enhanced the proliferation of hippocampal neurons in a dose-dependent manner, facilitated intracellular phosphorylation of cyclic AMP response element-binding protein (CREB), and upregulated the levels of pCREB and brain-derived neurotrophic factor (BDNF) in the mouse brain. Along with the result that both agents restored Aβ oligomer-induced synaptic loss and CREB phosphorylation, these findings suggest that elevated neurogenesis and synaptogenesis by BB and quercetin may share a common phosphorylated CREB-mediated signalling pathway[62]. Similarly, another animal study by Yin et al[63] also revealed that administration of BB (4 and 8 mg/kg) alleviated neuronal injury and apoptosis in the frontal cortex and hippocampal CA1 region in a rat AD model induced by Aβ25-35 and simultaneously improved memory and learning impairments in rats in the Morris water maze. In addition, the inhibition of TNF-α and Aβ1-40 expression was closely related to the action mechanisms of BB in this experimental model[63]. In an *in vitro* experiment using differentiated SH-SY5Y cells, Shi et al[64] found that BB promoted the secretion of α-secretase-cleaved soluble amyloid precursor protein (sAPPα, a by-product of non-amyloidogenic processing of APP) and lowered Aβ expression *via* a PI3K-dependent pathway, indicating that the PI3K- mediated effects of BB on APP processing may be regulated by intracellular APP trafficking. After prolonged incubation (12 h), the effects of BB were further strengthened due to PI3K-mediated upregulation of disintegrin and metalloprotease domain-containing protein 10 (an α-secretase candidate). Qin et al[65] recently reported that BB was capable of suppressing LPS-induced neuroinflammation and enhanced autophagy in LPS-treated BV-2 cells; however, treatment with BB elevated lincRNA-p21 levels, which in turn inhibited STAeT3 signalling. Further, *in vivo* experiments have also revealed the ability of BB to ameliorate learning and memory impairment in APP/PS1 AD mice. These results shed light on the pathogenesis of AD and may provide a new avenue for the treatment of AD with BB.

Potential Use of Ginkgolides and Bilobalide in Treating COVID-19 by Inhibiting SARS-CoV-2 Main Protease

In an attempt to use terpenoids from selected plant sources through bioinformatics tools for the inhibition of SARS-COV-2, Ullah et al[66] investigated almost 1,000 compounds for their inhibitory actions against 3-chymotrypsin-like cysteine protease (3CL^Pro), which is produced by the SARS-CoV-2 genome and is responsible for proteolytic processing of viral proteins during maturation. Exploratory investigation revealed that BB and GA interacted significantly with this protease and displayed high activity against flap regions of the protease[66]. In another study[67], 10 novel compounds, including ginkgolide M, were found to inhibit the 3CL^pro protein. The antiviral mechanisms of these compounds, albeit largely unknown, could be mediated through the suppression of the main protease of the coronaviruses and inhibition of the ion channels[68]. Nevertheless, ginkgolides and BB may be good candidates for treatment strategies against COVID-19 in the future.

Conclusions

An increasing number of studies have demonstrated a close relationship between AD and COVID-19. As two important bioactive components of GbE, ginkgolides and BB show neuroprotective effects in AD *via* multiple mechanisms such as anti-excitotoxicity, anti-inflammatory and anti-oxidative activities. Notably, ginkgolides and BB may also demonstrate antiviral properties against COVID-19 by inhibiting SARS-CoV-2 main protease. However, it remains to be determined whether long-term administration of pure ginkgolides or BB at potentially therapeutic levels are truly effective or toxic for the treatment of both AD and COVID-19. Therefore, more evidence, particularly from large-scale clinical trials, is needed to further understand the therapeutic efficacy and safety of these agents.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Ethics Approval
For this narrative review no ethics approval is needed.

Consent for Publication
Not required.

Availability of Data
The data used or analyzed during the current study are available from the corresponding author upon request.

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