

Research progress of EGCG in the treatment of Alzheimer's disease

Lin Meng¹, Changxing Liu², Lili Yuan², Xiang Chang^{3,*}

¹Cerebrovascular Disease of Integrated Traditional Chinese and Western Medicine, Shaanxi University of Traditional Chinese Medicine, Xi'an, China

²Cerebrovascular Disease, Shaanxi University of Traditional Chinese Medicine, Xi'an, China

³Xi'an Traditional Chinese Medicine Hospital, Xi'an, Shaanxi, China

*Corresponding author

Abstract: Alzheimer's disease (AD) is a degenerative disease of the nervous system. Mainly presenting with neuropsychiatric symptoms such as progressive memory impairment, cognitive dysfunction, personality changes, and language disorders^[1]. Also known as senile dementia, it is the leading cause of dementia in the elderly^[2]. Involved in 60-80% of all reports of dementia^[3]. As the global population ages, AD prevalence and mortality rates increase. It seriously affects the quality of life of the elderly and brings a heavy economic burden and severe social problems. The tea polyphenol epigallocatechin gallate (EGCG) has preventive and therapeutic effects on Alzheimer's disease by inhibiting the amyloid formation and aggregation, antioxidant, anti-inflammatory, metal chelating, binding cholinesterase, thereby reducing the degradation of acetylcholine, and repairing mitochondrial dysfunction. This article reviews the research on the mechanism of EGCG action on Alzheimer's disease.

Keywords: EGCG; Alzheimer's disease; amyloid antioxidant; anti-inflammatory

1. Introduction

Alzheimer's disease (AD) is a chronic progressive degenerative disease of the central nervous system and is the most common type of dementia in the elderly. In 2019, Alzheimer's disease and other forms of dementia were ranked by the World Health Organization as the seventh most common cause of death in the world^[4]. The onset of AD is usually insidious and progressive. Early-onset of mild cognitive impairment (MCI), daily living ability, such as personality and behavior changes, mental abnormalities, logical thinking, and comprehensive analysis of the ability to gradually damage, and eventually life-threatening. AD usually has an insidious onset and progressive progression. Pre-onset manifests mainly as mild cognitive impairment (MCI), gradual impairment of daily living abilities, such as the appearance of personality and behavioral changes, psychiatric abnormalities, diminished logical thinking and comprehensive analytical skills, and eventually life-threatening.

AD is a multifactorial disease characterized by progressive accumulation of A β protofibrils and abnormal tau proteins in the extracellular space and neurons, respectively, and loss of neurons and synapses in multiple brain regions, especially in the frontal cortex and hippocampus associated with them^[5-6]. Currently, only five drugs (tacrine, donepezil, carboplatin, galantamine, and memantine) are approved by the FDA for the treatment of AD. this disease is currently incurable, and the available drugs only control the symptoms but exhibit severe side effects^[7]. These drugs are based on a single-target strategy and focus on restoring neurotransmitter homeostasis. The search for disease-modifying AD therapies remains a pressing and unmet clinical need. Therefore, multi-targeted drugs with neuroprotective, anti-amyloid, and anti-inflammatory-antioxidant properties would effectively treat AD.

Epigallocatechin-3-gallate (EGCG) is a natural polyphenol isolated from tea, accounting for about 80% of tea polyphenols^[8]. Studies have found that EGCG can cross the blood-brain barrier and has antioxidant, anti-inflammatory, anti-tumor, and neuroprotective effects^[9]. Has a multi-targeted effect in the prevention of Alzheimer's disease. Therefore, this paper will summarize the research progress of EGCG in the prevention of Alzheimer's disease and elaborate on the multi-target prevention mechanism of EGCG in Alzheimer's disease.

2. EGCG and β -amyloid protein

The accumulation of amyloid β -protein ($A\beta$) is considered to be one of the primary pathogenesis of AD. $A\beta$ protein is produced from amyloid precursor protein (APP) by secretase degradation. Typically, the production and degradation of $A\beta$ are in balance. However, during AD pathology, the imbalance between $A\beta$ production and clearance leads to the aggregation of $A\beta$ and the formation of insoluble amyloid, which is deposited in brain tissue to form amyloid plaques^[10]. APP has two degradation pathways, mainly involving three secreted enzymes, α , β and γ . When APP is cleaved by α and γ secretases, a neuroprotective soluble extracellular fragment, sAPP α , is produced without $A\beta$, and this pathway is called the non-amyloid hydrolysis pathway. However, when APP is continuously hydrolyzed by β and γ secretases, polypeptides of different lengths, including $A\beta$ 1-40 and $A\beta$ 1-42 (collectively referred to as $A\beta$) are produced, and this pathway is called the amyloid hydrolysis pathway^[11]. These $A\beta$ monomers are aggregated and form soluble $A\beta$ oligomers, insoluble amyloid fibrils, and amyloid plaques, which exert neurotoxicity, lead to neuronal damage, and trigger AD^[12]. (Figure.1). Recent studies have shown that $A\beta$ can activate microglia, promote a chronic inflammatory state, and release pro-inflammatory cytokines, leading to neurodegeneration^[13]. Alastair^[14] showed that pro-inflammatory cytokines mediate $A\beta$ neurotoxicity through APP protein homeostasis. $A\beta$ also causes dysregulation of iron homeostasis and stimulates oxidative stress in microglia leading to AD^[13].

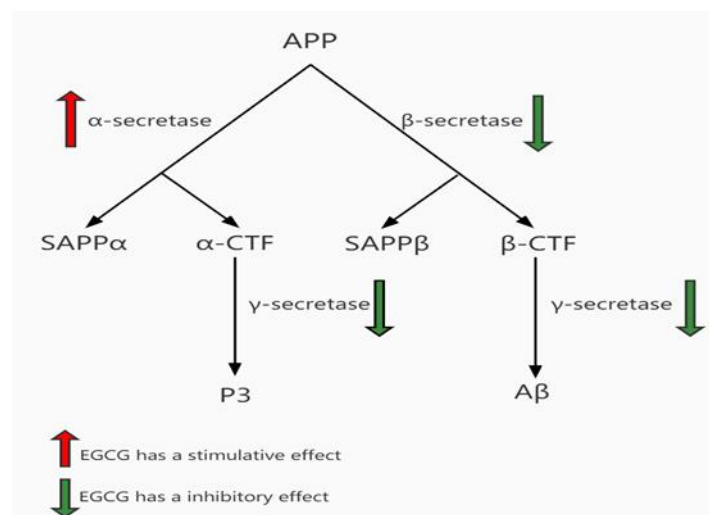


Figure 1: EGCG affecting the synthesis, degradation, and aggregation of $A\beta$

2.1. EGCG inhibits $A\beta$ production

Studies have shown that EGCG can interfere with $A\beta$ production. Intraperitoneal injection (20 mg/kg) of EGCG was found to significantly reduce $A\beta$ levels and $A\beta$ plaques in the brain in mice transfected with human APP mutant neuroblastoma cells. This study further demonstrated that EGCG promotes the release of soluble sAPP α and inhibits the production of $A\beta$ 1-40 and $A\beta$ 1-42 by up-regulating the expression of α -secretase^[15]. In addition, EGCG inhibits the expression of β -secretase^[16]. Lee et al.^[17] showed that EGCG was able to dose-dependently decrease β - and γ -secretase activities in the cerebral cortex and hippocampus of AD model mice, thereby reducing $A\beta$ levels, and elevated α -secretase activity was detected. EGCG reduces $A\beta$ production by activating the nuclear peroxisome receptor and inhibiting the expression of β -secretase^[18]. The β -site APP cleavage enzyme 1 (BACE1) is the rate-limiting enzyme in APP processing and $a\beta$ production, and negative regulation of BACE1 inhibits $a\beta$ production. EGCG reduces the enzymatic activity of BACE1 on APP by inhibiting its transcription and translation and ultimately reduces the production of $A\beta$ ^[19].

Regarding the chemical mechanism of EGCG- $A\beta$ interaction, it has been suggested that EGCG interferes with the aromatic hydrophobic core of $A\beta$, forming a nontoxic $A\beta$ oligomer^[20]. In conclusion, EGCG plays an essential role in the upstream regulation of $A\beta$ production. On the one hand, EGCG acts on three key rate-limiting enzymes of $A\beta$ production, namely α , β and γ secretase, to reduce the expression level of $A\beta$; on the other hand, EGCG reduces the expression of $A\beta$ by inhibiting APP degradation.

2.2. EGCG inhibits A β accumulation

It was found that the phenyl ring in the structure of polyphenols interacts with aromatic residues in the amyloid sequence and mediates the binding of these inhibitory groups to the center of the A β amino acid sequence, interfering with A β aggregation [16]. Analysis of transgenic AD mouse models showed a significant reduction in brain amyloid plaques, A β levels, and cognitive deficits after EGCG intervention treatment. In APPsw, Tg transgenic mice, Intraperitoneal injection and oral administration of EGCG reduced A β deposition in the mice. Six months after EGCG treatment, plaque load in the cingulate cortex, hippocampus, and internal olfactory cortex was reduced by 54%, 43% and 51%, respectively, and was accompanied by a reduction in A β 1-40 and a β 1-42 [21]. Bao et al. [22] further demonstrated that EGCG could reduce A β levels, inhibit plaque deposition and improve cognitive impairment through an APP/PS1 transgenic mouse model. Lee et al. [23] showed that EGCG could effectively disrupt the formation of A β 1-42 protofibrils, remodel performed protofibrils, and destabilize preformed A β 1-42 protofibrils. In addition, EGCG can prevent A β from accumulating into β -lamellar amyloid fibers by destroying intermolecular hydrogen bonds and hydrophobic bonds of A β [24].

2.3. EGCG promotes A β degradation

EGCG promotes A β degradation. The process of A β degradation in vivo is influenced by various peptidases and proteases, which are collectively known as A β -degrading proteases (A β DPs), although the types of A β -degrading enzymes and degradation mechanisms are not fully understood. Neutral endopeptidase (NEP) has been found to function as an A β degrading enzyme and is an essential rate-limiting enzyme in the degradation of A β 1-42, which degrades A β monomers and dimers. The levels of NEP are significantly decreased in the brains of AD patients, suggesting that NEP may play an essential role in this [25]. Xiang Chang [26] found that EGCG reduced the deposition of A β in the brains of SAMP8 mice and improved cognitive impairment in mice. They also found that the above effects of EGCG may be related to the fact that EGCG promoted the expression of NEP in the brain of aging mice and accelerated the degradation of A β . EGCG also affects the ERK pathway in astrocytes and the intracellular phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt)-mediated signaling pathway to promote the secretion of NEP and produce the effect of A β degradation [19]. In addition, EGCG promotes A β degradation by improving autophagy. EGCG can activate autophagy by regulating the mTOR signaling pathway in the mouse brain and up-regulating ULK1 protein expression; down-regulating LC3II/LC3I protein expression reduces autophagosome accumulation in the brain and facilitates the clearance of abnormal proteins [27]. In APP/PS1 transgenic mice, there was a large amount of A β deposition in the hippocampus, and the A β deposition in the hippocampus of transgenic mice was reduced after the administration of EGCG. It was shown that EGCG improved autophagy initiation, increased autophagic activity, and reduced autophagosome accumulation in the brain of APP/PS1 transgenic mice to improve autophagic dysfunction, which in turn reduced hippocampal A β deposition [27].

3. EGCG inhibits Tau phosphorylation and aggregation

Another typical alteration of AD is the aggregation of hyperphosphorylated Tau proteins to form neurofibrillary tangles (NFTs) that are not easily degraded by protein hydrolases, which accumulate in neurons, reduce the binding capacity of microtubules, disrupt the normal functioning of neuronal axons, interfere with the information transmission between neuronal synapses, cause structural and functional abnormalities in neuronal cells, and thus cause neurodegeneration [28]. In the SAMP8 mouse assay, EGCG was observed to reduce Tau protein hyperphosphorylation [29]. Nan S et al. [30] also confirmed the above view. Lin et al. [31] showed that EGCG treatment could inhibit the activation of glycogen synthase kinase-3 β (GSK-3 β) by reducing c-Abl kinase (Abelson tyrosine kinase), and through this pathway, it could enhance the hydrolysis of APP and thus reduce A β levels. Gsk-3 β has the function of promoting Tau Protein phosphorylation and can co-regulate Tau Protein with Protein Phosphatase-2A (PP-2A). And the latter can mediate Tau dephosphorylation [32]. EGCG inhibits the formation of NFTs from phosphorylated Tau proteins, also associated with the neuroautophagic system. Chesser et al. [33] showed that EGCG significantly increased the mRNA expression of critical autophagic bridging proteins NDP52 and p62, which in turn promoted the clearance of phosphorylated Tau proteins by the autophagic pathway.

4. Antioxidant

Oxidative stress is one of the pathogenesis of AD. Compared with ordinary people, the brains of AD

patients are more likely to produce large amounts of reactive oxygen species (ROS). One of the primary sources is the imbalance of the redox system due to A β and excess metal ions, which causes lipid and protein peroxidation and damage to neurons [34-35]. Excess ROS not only disrupts the normal physiological metabolism of the brain, its lipid peroxidation products, such as 4-Hydroxynonenal (HNE), can significantly increase the level of BACE and upregulate the expression level of A β [36]. The antioxidant effect can inhibit neuronal apoptosis mediated by oxidative stress response and play a positive role in the treatment of AD [37]. EGCG can act as a redox agent, and the phenolic hydroxyl groups in its molecular structure can effectively counteract free radical activity, scavenge reactive oxygen species (ROS), and exert significant antioxidant effects [38]. And oxidizes itself to highly stable compounds, thus scavenging a large number of harmful free radicals from the body [9]. Numerous studies have found that EGCG mainly reduces oxidative stress damage and improves the antioxidant and immune functions of the body by regulating antioxidant enzyme activity and protein kinase-related gene expression in vivo. EGCG can increase intracellular levels of reduced glutathione [39], upregulate the expression of P-38MAPK, nuclear factor Nrf2 and heme oxygenase 1 (HO-1) [40-41], and thus reduce ROS production. In vitro experiments have shown that EGCG reduces reactive oxygen species (ROS) levels, improves cell viability, and inhibits H₂O₂-induced apoptosis by phosphorylating the Akt and JNK pathways [9].

5. Anti-inflammatory

Inflammation is closely related to the pathology of AD and plays a crucial role in disease progression. A β deposition and tau protein formation in neurogenic fiber tangles cause abnormal microglial activation and release a series of pro-inflammatory factors such as COX-2, iNOS, and IL-1 β , resulting in progressive neuronal death [42-43]. Activation of glial cells, the release of inflammatory factors, and oxidative stress also promote A β production and precipitation [42-43]. AD patients have higher levels of pro-inflammatory cytokines; the levels of IL-6, SOLUBLE tumor necrosis factor receptor-1 (S TNF α -R1), and C-reactive protein (CRP) in blood were also higher [44]. It has been shown that EGCG can inhibit the expression of COX-2 and other pro-inflammatory factors by activating AMPK pathways in microglia and upregulating the expression of BDNF [45]. Zhong et al. [46] found that EGCG inhibits the activation of classical NLRP3 and non-classical caspase-11-dependent inflammasome by inhibiting the activation of the TLR4/NF- κ B pathway. And ultimately inhibits microglia-induced inflammatory response and neurotoxicity. In vitro experiments, EGCG reduced inflammation of mouse cells through the PI3K/Akt/mTOR pathway, suggesting that EGCG has potential therapeutic effects in regulating inflammation and controlling autoimmune diseases [47]. Cheng et al. [48] found that EGCG promotes endocytosis, thereby inhibiting lipopolysaccharide (LPS)-induced microglial activation and migration. It was further demonstrated in the APP/PS1 mouse assay that EGCG significantly attenuated microglial activation levels, decreased pro-inflammatory factor IL-1 β and increased anti-inflammatory factors IL-10 and IL-13, and a reduction in A β plaques was observed [22]. It indicates that the anti-inflammatory effect of tea polyphenol EGCG is mainly manifested in the regulation of inflammatory factors. Therefore, EGCG may be an effective agent against the development or progression of AD associated with neuroinflammation.

6. Chelate metal ions

Metal ions can promote the aggregation of A β , cause oxidative damage, and increase cytotoxicity, resulting in neuronal necrosis and brain tissue damage [49]. AD patients have higher concentrations of Cu²⁺, Zn²⁺, and Fe³⁺ in the brain [50], and EGCG has complexation for both Cu²⁺ and Fe³⁺ [51]. The chelation of iron by EGCG interferes with iron-induced degradation and inactivation of iron regulatory protein (IRP1/2), leading to a decrease in amyloid precursor protein (APP) [52]. It has been observed in electrochemistry and optics that EGCG inhibits the formation of Cu²⁺His complex by acting on Cu²⁺, thus reducing the formation of fiber [53]. EGCG also showed the ability to inhibit trivalent aluminum ion-induced A β 42 fibrillation and significantly reduced A β 42 toxicity by preventing further folding of A β 42 monomers [54]. EGCG not only chelates free metal ions but also regulates the aggregation of A β after metal binding. In the effect of EGCG-bound metal ions on the structure of A β , it was found that the addition of EGCG to Cu²⁺, Zn²⁺-bound A β generates free state A β aggregates, while structural A β aggregates are generated in metal-bound A β without the addition of EGCG [55]. These findings suggest that EGCG can compete with A β to bind metal ions, reduce APP production, prevent A β aggregation, and continue to inhibit A β aggregate binding after A β binding metal ions, thus preventing A β deposition and neurotoxicity.

Scholars have noted that the two structures that confer metal chelating properties on this compound include the *o*-3',4'-dihydroxy portion and the 4-keto, 3-hydroxy, or 4-keto and 5-hydroxy portion. These structures act as attachment sites for transition metals, neutralizing their activity by converting their active forms into redox inactive complexes to prevent oxidative damage to cells ^[56].

7. EGCG has a mitochondrial repair function

Mitochondrial dysfunction may be a predisposing factor for the progression of AD ^[57]. In physiology, mitochondria are the powerhouse of the cell, providing sufficient energy to maintain endogenous neuroprotective and repair mechanisms through oxidative phosphorylation. In contrast, damaged mitochondria are the main site of free radical generation, which directly triggers apoptosis through the cytoplasmic release of cytochrome c (Cyt C). Therefore, mitochondrial function is impaired, and neurons may also be damaged ^[58]. Studies have shown that excessive deposition of A β and abnormally phosphorylated tau proteins can affect mitochondrial function within neurons. Mitochondrial DNA (mtDNA) damage, mutations, or impaired metabolite transport may lead to A β oligomer or fibril formation and phosphorylated tau protein accumulation, further driving the course of AD disease ^[59-60]. Therefore, mitochondrial damage plays an important role in the pathogenesis of AD.

It has been shown that EGCG has mitochondrial reparability. EGCG treatment of A β PP/PS-1 double mutant transgenic AD mice restored mitochondrial respiration rate, mitochondrial membrane potential, ROS production, and ATP levels isolated from the hippocampus, cortex, and striatum by 50-85% ^[61]. A study of EGCG on mitochondrial damage after subarachnoid hemorrhage (SAH) found that EGCG protected mitochondria by inhibiting apoptosis induced by mitochondrial dysfunction. Meanwhile, EGCG exerts a neuroprotective mechanism by inhibiting overloaded [Ca²⁺]_i-induced mitochondrial dysfunction and imbalance of the mitochondrial fusion-fission cycle ^[62]. In vivo, EGCG also promotes the production of skeletal muscle mitochondrial DNA in SAMP8 mice and repairs damaged skeletal muscle mitochondrial DNA ^[63]. In a neural precursor cell model of Down syndrome (DS), EGCG exhibits neuroprotective activity and promotes mitochondrial bioenergetics, mitochondrial biogenetic programs, and cell proliferation through activation of the AMPK-PGC-1 α -Sirt1 signaling pathway ^[64]. In addition, EGCG inhibits mitochondria-mediated apoptosis by regulating VDAC and cytochrome C. VDAC is a vital channel located on the outer mitochondrial membrane and is a regulator of mitochondrial regulation of apoptosis. Studies have reported that VDAC regulates the expression of cytochrome c, which is an essential pharmacological drug target for AD research in recent years ^[65].

8. EGCG inhibits acetylcholine degradation

One of the etiologies of AD is due to a dysregulation of the cholinergic system in the brain with decreased levels of acetylcholine (ACh), resulting in reduced signaling ^[66]. ACh acts as a neurotransmitter that works specifically on various types of choline receptors, and its cleavage enzymes are mainly Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) ^[67]. A molecular docking study showed that EGCG inhibits AChE and BuChE and enhances cholinergic neurotransmission by prolonging the duration of action ^[68]. Okello et al. ^[69] isolated four catechins EC, EGC, ECG, and EGCG from green tea and found that EGCG was the only compound with statistically significant competitive inhibition of AChE and BuChE and measured half-inhibitory concentration (IC₅₀) values of 0.0148 μ mol/mL and 0.0251 μ mol/mL, respectively. In addition, cholinesterase inhibitors may affect amyloid processing in AD ^[68]. Therefore, cholinesterase inhibitors are recommended as standard agents for the treatment of AD.

9. Bioavailability development of EGCG

As mentioned above, EGCG is one of the favorable candidates for preventing Alzheimer's disease, but the bioavailability of EGCG is poor, and it is difficult to reach the concentration of optimal efficacy. And the half-life is short, and the drug action time is short. In addition, the absorption rate of EGCG orally is also affected by other foods and drugs taken together [9]. So, it is crucial to improve the bioavailability of EGCG. Current developments to enhance the efficacy of EGCG fall into three categories: combination drugs, nanotechnology, and EGCG precursor drugs. The combined effect plays a role in increasing the bioavailability of EGCG. Mori et al. ^[70] suggested that the combined effect of EGCG and ferulic acid was superior to that of the inhibition of A β levels, deposition, and the ability to regulate the associated secretory enzymes in the mouse brain alone. The combined effect of EGCG and

fish oil showed that compared with EGCG alone, fish oil could improve the bioavailability of EGCG [71]. In nanotechnology, compared with traditional drug delivery methods, the nanoparticle drug delivery system has the advantages of strengthening drug stability and bioavailability, enhancing drug targeting. Cano et al. [72] developed dual drug-loaded polyethylene glycolized PLGA nanoparticles (EGCG/AA NPs) that showed higher stability, promoted increased synaptophysin, and reduced neuroinflammation and A β activity in mice. Smith et al. [73] developed nano-lipid EGCG particles that increased α -secretase levels in SweAPP N2a cells by 91% and were twice as effective as oral free EGCG. Yang Peng et al. [74] applied EGCG to the nano delivery system and developed NP/EGCG (RD2-NP/EGCG) that modified the surface of the RD2 peptide. It was found that RD2-NP/EGCG concentrated in the hippocampus and cerebral cortex significantly reduced the levels of inflammatory factors TNF- α and IL-1 β in the brain of AD model mice, repaired neuronal injury, and improved spatial memory disorder without organ toxicity. In addition to changing the way EGCG is administered, it can be chemically modified to form EGCG precursor drugs, and This allows the drug to be released by the action of specific enzymes when it reaches a particular site, thus avoiding damage during transport in the body and maximizing its effect. Hey et al. [75] found that pre-EGCG (EGCG octaacetate) can target ERCC1/XPF activity and enhances the anticancer efficacy of cisplatin in vitro and in vivo. Jiao et al. [76] found that EGCG prodrug (pro-EGCG) alleviated choroidal neovascularization (CNV) leakage and reduced choroidal neovascularization area in mice by inhibiting HIF-1 α /VEGF/VEGFR2 pathway and M1-type macrophage/microglia polarization and reducing choroidal endothelial cell proliferation, migration and tube formation, Suggest a potential new approach for the treatment of AMD. Therefore, future researchers should focus more on developing novel EGCG vectors or precursor drugs with selective and specific targeting of disease tissues.

10. Conclusion and Outlook

The pathogenic factors of Alzheimer's disease are complex, interrelated, and mutually promoting, involving a variety of cellular and molecular mechanisms. For example, A β deposition and phosphorylated Tau proteins promote each other, which in turn leads to cholinergic damage, oxidative stress, inflammatory damage, and impaired endoplasmic reticulum mitochondrial function, and these downstream etiologies in turn, feedback to promote A β deposition and the production of NFTs, exacerbating the course of AD. EGCG, as a tea extract, is a natural antioxidant with low biological toxicity and multiple targets. EGCG can intervene in AD and is a potential preventive drug for AD. EGCG reduces the level of A β production by decreasing APP production and regulating alpha, beta, and gamma secretory enzymes to reduce the level of A β activity. Inhibits A β deposition by breaking A β hydrogen and hydrophobic bonds; it also chelates metal ions to inhibit A β oligomerization. Promotes clearance of phosphorylated Tau proteins by the autophagic pathway by affecting the kinase and phosphatase of Tau and thereby interfering with Tau phosphorylation. Binding cholinesterase thereby reducing the degradation of acetylcholine; resistance to oxidative stress and inflammation; repair of mitochondrial disorders, etc. It indicates that EGCG can affect and regulate various AD causes and protect the brain's nervous system in a multi-targeted manner, thus preventing Alzheimer's disease. Combined with the current nanotechnology and the depth of Alzheimer's disease research, EGCG will have a broad application prospect.

References

- [1] 2020 Alzheimer's disease facts and figures. *Alzheimer's Dement.* 2020;10.1002/alz.12068.
- [2] Goldfarb D, Sheard S, Shaughnessy L, Atri A. Disclosure of Alzheimer's Disease and Dementia: Patient- and Care Partner-Centric Decision-Making and Communication. *J Clin Psychiatry.* 2019; 80(2): MS18002BR1C.
- [3] Zhu Z, Liu Y, Li X, et al. GPNMB mitigates Alzheimer's disease and enhances autophagy via suppressing the mTOR signal. *Neurosci Lett.* 2021;136300.
- [4] World Health Organization WHO - The top 10 causes of death. Available online: <http://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death> (accessed on Jan 6, 2021).
- [5] Lane CA, Hardy J, Schott JM. Alzheimer's disease. *Eur J Neurol.* 2018; 25(1): 59-70. doi:10.1111/ene.13439
- [6] Busche MA, Hyman BT. Synergy between amyloid- β and tau in Alzheimer's disease. *Nat Neurosci.* 2020; 23(10): 1183-1193.
- [7] Gonçalves PB, Sodero ACR, Cordeiro Y. Green Tea Epigallocatechin-3-gallate (EGCG) Targeting Protein Misfolding in Drug Discovery for Neurodegenerative Diseases. *Biomolecules.* 2021; 11(5): 767.
- [8] Pervin M, Unno K, Takagaki A, Isemura M, Nakamura Y. Function of Green Tea Catechins in the

- Brain: Epigallocatechin Gallate and its Metabolites. *Int J Mol Sci.* 2019; 20(15): 3630.
- [9] ZENG Lu, ZHANG Shuai, AI Jing. *Research Progress of Epigallocatechin Gallate [J]. Journal of Neuropharmacology*, 2021, 11(1): 38-56.
- [10] Salminen A, Kaarniranta K, Kauppinen A, et al. *Impaired autophagy and APP processing in Alzheimer's disease: The potential role of Beclin 1 interactome. Prog Neurobiol.* 2013; 106-107: 33-54.
- [11] LA FERIA E, GREEN I, ODDO S. *Intracellular amyloid- β in Alzheimer's disease [J]. Nature RevNeurosci*, 2007, 8(7): 499-509.
- [12] Yu H, Wu J. *Amyloid- β : A double agent in Alzheimer's disease?. Biomed Pharmacother.* 2021; 139: 111575.
- [13] Alastair F, Alshammari MA, Alasmari AF, et al. *Neuroinflammatory Cytokines Induce Amyloid Beta Neurotoxicity through Modulating Amyloid Precursor Protein Levels/Metabolism. Biomed Res Int.* 2018; 2018: 3087475.
- [14] Payne A, Nahashon S, Taka E, et al. *Epigallocatechin-3-Gallate (EGCG): New Therapeutic Perspectives for Neuroprotection, Aging, and Neuroinflammation for the Modern Age. Biomolecules.* 2022; 12(3): 371.
- [15] Rezai-Zadeh K, Shytle D, Sun N, et al. *Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci.* 2005; 25(38): 8807-8814.
- [16] Fernandes L, Cardim-Pires TR, Fogel D, Palthano FL. *Green Tea Polyphenol Epigallocatechin-Gallate in Amyloid Aggregation and Neurodegenerative Diseases. Front Neurosci.* 2021; 15: 718188.
- [17] Lee J W, Lee Y K, Ban J O, et al. *Green tea (-)-epigallocatechin-3-gallate inhibits beta-amyloid-induced cognitive dysfunction through modification of secretase activity via inhibition of ERK and NF-kappaB pathways in mice[J]. J Nutr*, 2009, 139 (10):1987-93.
- [18] Zhang ZX, Li YB, Zhao RP. *Epigallocatechin Gallate Attenuates β -Amyloid Generation and Oxidative Stress Involvement of PPAR γ in N2a/APP695 Cells. Neurochem Res.* 2017; 42(2): 468-480.
- [19] Polito CA, Cai ZY, Shi YL, et al. *Association of Tea Consumption with Risk of Alzheimer's Disease and Anti-Beta-Amyloid Effects of Tea. Nutrients.* 2018; 10(5): 655.
- [20] Gon galves PB, Sodero ACR, Cordeiro Y. *Green Tea Epigallocatechin-3-gallate (EGCG) Targeting Protein Misfolding in Drug Discovery for Neurodegenerative Diseases. Biomolecules.* 2021; 11(5): 767.
- [21] Rezai-Zadeh K, Arendash GW, Hou H, et al. *Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. Brain Res.* 2008; 1214: 177-187.
- [22] Bao J, Liu W, Zhou HY, et al. *Epigallocatechin-3-gallate Alleviates Cognitive Deficits in APP/PS1 Mice. Curr Med Sci.* 2020; 40(1): 18-27.
- [23] Lee SB, Choi EH, Jeong KH, Kim KS, Shim SM, Kim GH. *Effect of catechins and high-temperature-processed green tea extract on scavenging reactive oxygen species and preventing A β 1-42 fibrils' formation in brain microvascular endothelium. Nutr Neurosci.* 2020; 23(5): 363-373.
- [24] Wang SH, Liu FF, Dong XY, Sun Y. *Thermodynamic analysis of the molecular interactions between amyloid beta-peptide 42 and (-)-epigallocatechin-3-gallate. J Phys Chem B.* 2010;114(35): 11576-11583.
- [25] Salim S El-Amouri, Hong Zhu, Jin Yu, et al. *Nepriylsin: An enzyme candidate to slow the progression of Alzheimer's disease [J]. Am J Pathol*, 2008, 172 (5): 1342-1354.
- [26] Xiang Chang, Cui-ping Rong, Yun-bo Chen, et al. *(-)-epigallocatechin-3-gallate attenuates cognitive deterioration in Alzheimer's disease model mice by upregulating neprilysin expression [J]. Experimental Cell Research*, 2015, 334 (1): 136-145.
- [27] Chengyan Sun, Ning JIA, Kun Han. *Effects of epigallocatechin gallate on cognitive function and hippocampal autophagy in APP/PS1 transgenic mice [J]. Chinese Pharmacological Bulletin*, 2020, 36 (04): 539-543.
- [28] Drummond E, Pires G, MacMurray C, et al. *Phosphorylated tau interactome in the human Alzheimer's disease brain. Brain.* 2020; 143(9): 2803-2817.
- [29] Guo Y, Zhao Y, Nan Y, et al. *(-)-Epigallocatechin-3-gallate ameliorates memory impairment and rescues the abnormal synaptic protein levels in the frontal cortex and hippocampus in a mouse model of Alzheimer's disease [J]. Neuroreport*, 2017, 28 (10): 590-597.
- [30] Nan S, Wang P, Zhang Y, Fan J. *Epigallocatechin-3-Gallate Provides Protection Against Alzheimer's Disease-Induced Learning and Memory Impairments in Rats. Drug Des Devel Ther.* 2021; 15: 2013-2024.
- [31] Lin CL, Chen T F, Chiu M J, et al. *Epigallocatechin gallate (EGCG) suppresses beta-amyloid-induced neurotoxicity through inhibiting c-Abl/FE65 nuclear translocation and GSK3 beta activation[J]. Neurobiol Aging*, 2009, 30 (1): 81-92.
- [32] Xu Y H, Chen Y, Zhang P, et al. *Structure of a protein phosphatase 2A holoenzyme: Insights into B55-mediated Tau dephosphorylation [J]. Molecular Cell*, 2008, 31(6): 873-85.

- [33] Chesser AS, Ganeshan V, Yang J, et al. Epigallocatechin-3-gallate enhances clearance of phosphorylated tau in primary neurons. *Nutr Neurosci*. 2016; 19(1): 21-31.
- [34] Uttara B, Singh AV, Zamboni P, et al. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options [J]. *Curr Neuropharmacol*, 2009, 7 (1): 65-74.
- [35] Butterfield D A, Castagna A, Lauderback CM, et al. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death[J]. *Neurobiol Aging*, 2002, 23 (5): 655-64.
- [36] Tamagno E, Bardini P, Obbili A, et al. Oxidative stress increases expression and activity of BACE in NT2 neurons[J]. *Neurobiol Dis*, 2002, 10 (3): 279-88.
- [37] Plascencia-Villa G, Perry G. Preventive and Therapeutic Strategies in Alzheimer's Disease: Focus on Oxidative Stress, Redox Metals, and Ferroptosis. *Antioxid Redox Signal*. 2021;34(8):591-610.
- [38] Teng Y, Zhao J, Ding L, et al. Complex of EGCG with Cu(II) Suppresses Amyloid Aggregation and Cu(II)-Induced Cytotoxicity of α -Synuclein. *Molecules*. 2019;24(16):2940.
- [39] Gyanendra Singh, Riddhi Thaker, Anupama Sharma, et al. Therapeutic effects of biochanin a, phloretin, and epigallocatechin-3-gallate in reducing oxidative stress in arsenic-intoxicated mice [J]. *J Biochem Mol Toxicol*, 2021, 28(16): 20517-20536.
- [40] Smith RE, Tran K, Smith CC, McDonald M, Shejwalkar P, Hara K. The Role of the Nrf2/ARE Antioxidant System in Preventing Cardiovascular Diseases. *Diseases*. 2016; 4(4): 34.
- [41] Wang J, Jia R, Celi P, et al. Green tea polyphenol epigallocatechin-3-gallate improves the antioxidant capacity of eggs. *Food Funct*. 2020;11(1): 534-543.
- [42] Bostancıklıoğlu M. An update on the interactions between Alzheimer's disease, autophagy and inflammation. *Gene*. 2019; 705: 157-166.
- [43] Walker KA, Ficek BN, Westbrook R. Understanding the Role of Systemic Inflammation in Alzheimer's Disease. *ACS Chem Neurosci*. 2019; 10(8): 3340-3342.
- [44] WALKER K A, FICEK BN, WESTBROOK R. Understanding the role of systemic inflammation in Alzheimer's disease [J]. *ACS Chemical Neuroscience*, 2019, 10(8): 3340-3342.
- [45] Sheng-Wei Lai, Jia-Hong Chen, Hsiao-Yun Lin, et al. Regulatory effects of neuroinflammatory responses through brain-derived neurotrophic factor signaling in microglial cells [J]. *Molecular Neurobiology*, 2018, 55 (9): 7487-7499.
- [46] Zhong X, Liu M, Yao W, et al. Epigallocatechin-3-Gallate Attenuates Microglial Inflammation and Neurotoxicity by Suppressing the Activation of Canonical and Noncanonical Inflammasome via TLR4/NF- κ B Pathway. *Mol Nutr Food Res*. 2019; 63(21): e1801230.
- [47] Peairs A, Dai R, Gan L, et al. Epigallocatechin-3-gallate (EGCG) attenuates inflammation in MRL/LPR mouse mesangial cells. *Cell Mol Immunol*. 2010; 7(2): 123-132.
- [48] Cheng CY, Barro L, Tsai ST, et al. Epigallocatechin-3-Gallate-Loaded Liposomes Favor Anti-Inflammation of Microglia Cells and Promote Neuroprotection. *Int J Mol Sci*. 2021; 22(6): 3037.
- [49] Wang L, Yin YL, Liu XZ, et al. Current understanding of metal ions in the pathogenesis of Alzheimer's disease. *Transl Neurodegener*. 2020; 9: 10.
- [50] Savelieff MG, Lee S, Liu Y, Lim MH. Untangling amyloid- β , tau, and metals in Alzheimer's disease. *ACS Chem Biol*. 2013; 8(5): 856-865.
- [51] Zwolak I. Epigallocatechin Gallate for Management of Heavy Metal-Induced Oxidative Stress: Mechanisms of Action, Efficacy, and Concerns. *Int J Mol Sci*. 2021;22(8):4027.
- [52] Mandel SA, Weinreb O, Amit T, Youdim MB. Molecular mechanisms of the neuroprotective/neurorescue action of multi-target green tea polyphenols. *Front Biosci (Schol Ed)*. 2012; 4(2): 581-598.
- [53] Zhang B, Cheng XR, da Silva IS, et al. Electroanalysis of the interaction between (-)-epigallocatechin-3-gallate (EGCG) and amyloid- β in the presence of copper. *Metallomics*. 2013; 5(3): 259-264.
- [54] Singh NA, Mandal AKA, Khan ZA. Inhibition of Al(III)-Induced A β 42 Fibrillation and Reduction of Neurotoxicity by Epigallocatechin-3-Gallate Nanoparticles. *J Biomed Nanotechnol*. 2018; 14(6): 1147-1158.
- [55] Hyung S J, Detoma A S, Brender J R, et al. Insights into anti-amyloidogenic properties of the green tea extract (-)-epigallocatechin-3-gallate toward metal-associated amyloid-beta species[J]. *Proc Natl Acad Sci U S A*, 2013, 110 (10): 3743-8.
- [56] Singh NA, Mandal AK, Khan ZA. Potential neuroprotective properties of epigallocatechin-3-gallate (EGCG). *Nutr J*. 2016; 15(1): 60.
- [57] Sharma C, Kim S, Nam Y, Jung UJ, Kim SR. Mitochondrial Dysfunction as a Driver of Cognitive Impairment in Alzheimer's Disease. *Int J Mol Sci*. 2021; 22(9): 4850.
- [58] Camilleri A, Zarb C, Caruana M, et al. Mitochondrial membrane permeabilization by amyloid aggregates and protection by polyphenols. *Biochim Biophys Acta*. 2013; 1828(11): 2532-2543.

- [59] Abyadeh M, Gupta V, Chitranshi N, et al. Mitochondrial dysfunction in Alzheimer's disease - a proteomics perspective. *Expert Rev Proteomics*. 2021; 18(4): 295-304.
- [60] Weidling IW, Swerdlow RH. Mitochondria in Alzheimer's disease and their potential role in Alzheimer's proteostasis. *Exp Neurol*. 2020; 330: 113321.
- [61] Dragicevic N, Smith A, Lin X, et al. Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. *J Alzheimers Dis*. 2011; 26(3): 507-521.
- [62] Chen Y, Chen J, Sun X, et al. Evaluation of the neuroprotective effect of EGCG: a potential mechanism of mitochondrial dysfunction and mitochondrial dynamics after subarachnoid hemorrhage. *Food Funct*. 2018;9(12):6349-6359.
- [63] Liu HW, Chan YC, Wang MF, et al. Dietary (-)-Epigallocatechin-3-gallate Supplementation Counteracts Aging-Associated Skeletal Muscle Insulin Resistance and Fatty Liver in Senescence-Accelerated Mouse. *J Agric Food Chem*. 2015;63(38):8407-8417.
- [64] Valenti D, de Bari L, de Rasmio D, et al. The polyphenols resveratrol and epigallocatechin-3-gallate restore the severe impairment of mitochondria in hippocampal progenitor cells from a Down syndrome mouse model. *Biochim Biophys Acta*. 2016;1862(6):1093-1104.
- [65] Ayyalasomayajula N, Bandaru LJM, Chetty CS, et al. Mitochondria-Mediated Moderation of Apoptosis by EGCG in Cytotoxic Neuronal Cells Induced by Lead (Pb) and Amyloid Peptides [published online ahead of print, 2021 Nov 19]. *Biol Trace Elem Res*. 2021;10.1007/s12011-021-02959-w.
- [66] Colangelo C, Shichkova P, Keller D, Markram H, Ramaswamy S. Cellular, Synaptic and Network Effects of Acetylcholine in the Neocortex. *Front Neural Circuits*. 2019; 13: 24.
- [67] Greig NH, Utsuki T, Yu Q, et al. A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase[J]. *Curr Med Res Opin*, 2001, 17 (3): 159-65.
- [68] Ali B, Jamal QM, Shams S, et al. In Silico Analysis of Green Tea Polyphenols as Inhibitors of AChE and BChE Enzymes in Alzheimer's Disease Treatment. *CNS Neurol Disord Drug Targets*. 2016; 15(5): 624-628.
- [69] Okello EJ, Mather J. Comparative Kinetics of Acetyl- and Butyryl-Cholinesterase Inhibition by Green Tea Catechins/Relevance to the Symptomatic Treatment of Alzheimer's Disease. *Nutrients*. 2020; 12(4): 1090.
- [70] Mori T, Koyama N, Tan J, et al. Combined treatment with the phenolics (-)-epigallocatechin-3-gallate and ferulic acid improves cognition and reduces Alzheimer-like pathology in mice [J]. *J Biol Chem*, 2019, 294 (8): 2714-2731.
- [71] Giunta B, Hou H, Zhu Y, et al. Fish oil enhances anti-amyloidogenic properties of green tea EGCG in Tg2576 mice [J]. *Neurosci Lett*, 2010, 471 (3): 134-8.
- [72] Cano A, Ettcheto M, Chang JH, et al. Dual-drug loaded nanoparticles of Epigallocatechin-3-gallate (EGCG)/Ascorbic acid enhance therapeutic efficacy of EGCG in a APP^{swe}/PS1^{dE9} Alzheimer's disease mice model. *J Control Release*. 2019; 301: 62-75.
- [73] Smith A, Giunta B, Bickford P C, et al. Nanolipidic particles improve the bioavailability and alpha-secretase inducing ability of epigallocatechin-3-gallate (EGCG) for the treatment of Alzheimer's disease[J]. *Int J Pharm*, 2010, 389 (1-2): 207-12.
- [74] Yang Peng, Guo Qian, Xu Shu-ting, et al. Constructing Brain A β -Targeting Nanoparticles Loaded with EGCG for Treating Alzheimer's Disease in Mice[J]. *Journal of Sichuan University (Medical Science Edition)*, 2021, 52 (04): 605-611.
- [75] Hey JR, Arora S, Zhang H, et al. Targeting the DNA Repair Endonuclease ERCC1-XPF with Green Tea Polyphenol Epigallocatechin-3-Gallate (EGCG) and Its Prodrug to Enhance Cisplatin Efficacy in Human Cancer Cells. *Nutrients*. 2018;10(11):1644.
- [76] Xu J, Tu Y, Wang Y, et al. Prodrug of epigallocatechin-3-gallate alleviates choroidal neovascularization via down-regulating HIF-1 α /VEGF/VEGFR2 pathway and M1 type macrophage/microglia polarization. *Biomed Pharmacother*. 2020; 121: 109606.