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Extracellular vesicle–based drug delivery system boosts phytochemicals' therapeutic effect for neurodegenerative diseases

Zhenzhu Zhu[1,*], Liuyue Liao[1], Hongzhi Qiao[2]

Abstract

Neurodegenerative diseases (NDs) are a major threat to the elderly, and efficient therapy is rarely available. A group of phytochemicals has been shown to ameliorate NDs; however, poor stability, low bioavailability, and reduced drug accumulation in brain tissue limit their application in NDs. Therefore, a targeted drug delivery system is a feasible treatment strategy for NDs. Extracellular vesicles (EVs) possess many favorable bioactivities and are excellent carriers for targeting brain tissue. This review summarizes EVs as novel phytochemical carriers in ND therapy. First, we discuss the current challenges of ND therapy and the therapeutic effects of phytochemicals for NDs. Second, we highlight the ability of EVs to cross the blood-brain barrier and act as drug carriers to enhance the therapeutic efficacy of drugs for NDs. Finally, encapsulation strategies for phytochemicals in EVs are particularly reviewed, as they are critical for obtaining high loading efficacy and stable drug delivery systems. This review provides new insights into EV-based drug delivery systems for improving the therapeutic effect of phytochemicals for ND treatment. Therefore, the release rate and pharmacokinetics of phytochemicals should be well controlled to ensure the therapeutic efficacy of phytochemical-loaded EVs in the brain.

Keywords: Bioavailability, Brain-targeting, Extracellular vesicle-based drug delivery system, Neurodegenerative diseases, Phytochemicals

Introduction

Neurodegenerative diseases (NDs) are becoming a serious threat owing to the growing elderly population worldwide, and the social burden has increased in recent decades. The four major types of NDs are Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and amyotrophic lateral sclerosis (ALS). According to a 2022 report[1], approximately 6.2 million Americans have AD, and more than 10 million people worldwide are living with PD as of 2020. In the future, the number of patients with NDs will increase significantly. The pathogenesis of NDs is complex and includes genetic, environmental, and other factors[2]. In the progression of NDs, neuroinflammation, aggregation of misfolded proteins, oxidative stress, inclusion body formation, and mitochondrial dysfunction are considered major causes in the brain[3]. NDs occur when nerve cells in the brain or peripheral nervous system lose function, and these diseases affect bodily functions, such as balance, movement, talking, breathing, and heart function.

Intensive efforts have been made to develop new drugs to treat NDs. To date, some drugs have been approved for the treatment of NDs in clinical settings; for example, donepezil, memantine, galantamine, and rivastigmine are used for AD therapy; carbidopa, levodopa, pramipexole, ropinirole for PD therapy; tetrabenazine for HD therapy; and risdiplam for ALS therapy[4]. Treatment may relieve some physical or mental symptoms associated with NDs; however, there is currently no treatment to slow disease progression and no known cure. This situation requires clinicians to improve their understanding of the causes of NDs and develop new treatment and prevention strategies.

Phytochemicals extracted from herbs such as *Panax ginseng*, *Eucommia ulmoides*, *Coptis chinensis*, *Ginkgo biloba L. Magnolia officinalis*, *Camellia sinensis*, and *Morus alba L.* are beneficial to human health[5]. Dietary fruits and vegetables also contain phytochemicals such as *Dioscoreae rhizoma*, *Curcuma longa*, *Zingiber officinale*, *Garcinia parvifolia*, *Vitis vinifera*, and berries, which provide nutrition[6]. Natural phytochemicals from herbs or edible plants have been identified and characterized as having therapeutic potential for NDs in recent years, and they may be less toxic than novel synthetic drugs[7]. Their mechanisms of action in the progression of NDs are complicated and include anti-oxidative, anti-inflammatory, anti-apoptotic, anti-aggregation, and mitochondrial protection effects. In addition, the role of the gut microflora in the central nervous system (CNS) mediates the therapeutic efficacy of phytochemicals[8]. However, the solubility, bioavailability, targeting capability, and effective concentrations of phytochemicals in the brain are major concerns[9].

[1] College of Food Science and Engineering, Nanjing University of Finance and Economics/Collaborative Innovation Center for Modern Grain Circulation and Safety, Nanjing, China; [2] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, China

*Corresponding author. Zhenzhu Zhu, College of Food Science and Engineering, Nanjing University of Finance and Economics, Nanjing, 210023, China, E-mail: 9120191020@nufe.edu.cn.

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Therefore, recent studies have focused on drug delivery systems to solve these problems. Examples of nanocarriers include liposomes, micelles, metal-based nanoparticles, and extracellular vesicles (EVs). EVs are more promising vehicles than traditional nano drug carriers for delivering drugs into the brain, owing to their biosafety, ability to cross the blood-brain barrier (BBB), targeted delivery, and low immunogenicity[10]. This review focuses on the application of phytochemical-loaded EVs in ND therapy. The literature review was conducted to find all published systematic reviews on the extracellular vesicle-based drug delivery system improving phytochemicals intervention in the neurodegenerative diseases. A comprehensive systematic literature was searched in the Web of Science, PubMed, SciFinder, Google scholar, and China National Knowledge Infrastructure. The information on clinical trials and ND therapeutics were collected from the website of Food and Drug Administration (FDA) of the United States and ClinicalTrials.gov. The literature searches most cover the period from January 2012 to September 2022.

Challenges of ND therapy

In recent years, there have been many challenges in developing therapeutic strategies for NDs. First, the exact targets of NDs remain controversial. AD is caused by the progressive formation of senile plaques and neurofibrillary tangles in the cerebral cortex, as well as neuronal and synaptic loss. The expression of amyloid precursor protein (APP), acetylcholinesterase (AChE) activity, and excess aggregated β-amyloid (Aβ) peptides initiate the pathogenic cascade, including the propagation of microtubule-associated tau aggregation throughout the brain[11]. PD is caused by the progressive death of neurons in the pars compacta region of the brain and alpha-synuclein (α-syn) aggregation, which causes a decrease in dopamine synthesis and Lewy body formation[12]. HD is caused by the expression of mutant huntingtin protein (mHTT) with an abnormal number of glutamine repeats in its N-terminus and is characterized by intracellular mHTT aggregates in the brain[13]. ALS is caused partly by neuronal cytoplasmic inclusions of the tar-deoxyribonucleic acid binding protein-43 (TDP-43) and superoxide dismutase 1 (SOD1)[14]. Great progress has been made in the research field of NDs; however, the key underlying molecular defects or pathways are unidentified. Recent studies have discovered that autolysosome acidification declines in neurons before Aβ deposition[15], which is subversive, and Aβ might not be the precise target of AD. In other words, there are no easily measurable biomarkers to predict patient progression. Second, the BBB is a major obstacle. Brain endothelial cells, astrocyte end-feet, and pericytes are found in the brain microvasculature between the blood circulation system and the CNS[16]. Endothelial tight junctions form a physical barrier, and membrane transporters in the efflux system form a transport barrier[17]. Drugs with molecular weights less than 400 Da and hydrogen bond numbers less than eight are allowed to pass through the BBB[18]. Regrettably, many drugs fail to reach the treatment site through the BBB because their predicted lipophilicity (log P) or lipophilic permeability coefficient (log D) is much higher than the actual CNS permeability of lipophilic drugs. Therefore, drugs for ND therapy that reach clinical trial stages are limited, especially for small molecule and macromolecule drugs (peptides, antibodies, and small interfering ribonucleic acid)[19].

Third, the therapeutic efficacy of approved drugs is not as expected. First-generation anti-AD drugs inhibit AChE and the *N*-methyl-D-aspartic acid receptor[20]. However, they did not significantly improve the state of AD but only slowed its progression[21]. Subsequently, β-secretase (BACE-1) blockers were developed, which decreased Aβ levels in neurons and glial cells. BACE-1 blockers, when used early in the course of AD, prevent disease progression[22]. In 2019, GV-971 received conditional marketing approval in China to improve cognitive function in mild to moderate AD by restoring the gut microbial profile to normal and reducing brain immune cell infiltration and inflammation[23]. In 2021, aducanumab was approved in the United States for AD treatment[24]. However, the clinical use of GV-971 and aducanumab has recently been terminated owing to complicated reasons and problems. Immunotherapies against Aβ and tau proteins are in the advanced stages of clinical trials and have the potential to block the progression of AD[25]. In addition, preclinical models do not accurately reflect the pathogenesis of NDs in humans[26], which is a challenge in clinical drug development.

Phytochemicals for ND therapy

Phytochemicals are bioactive compounds enriched in plants. Based on their chemical structure, they are classified as phenolic compounds, terpenes, carotenoids, alkaloids, nitrogen-containing compounds, and organosulfur compounds[27]. Numerous studies have demonstrated that phytochemicals can protect neurons from dysfunction and pave the way for ND therapy[28]. Typical compounds are resveratrol, curcumin, quercetin (Que), cyanidin-3-O-glucoside (C3G), epigallocatechin-3-galate (EGCG), berberine (Ber), and other bioactive compounds from plants (Figure 1). Most of these phytochemicals could directly interact with aggregation-prone proteins in different NDs, such as Aβ, tau, α-syn, polyglutamine, SOD1, and TDP-43, or indirectly interact with pathways related to ND progression *in vivo*. Anti-oxidative, anti-inflammatory, and anti-apoptotic effects and mitochondrial protection are closely related to the neuroprotective potential of phytochemicals (Table 1).

In AD, resveratrol exhibits anti-inflammatory effects by inhibiting nucleotide-binding and oligomerization domain-like receptor protein 3 (NLRP3) inflammasome activation and interleukin-1 beta (IL-1β) secretion in the CNS[29]. Curcumin administration reduces oxidative stress, which helps to destroy β-amyloid plaques and slows the progression of AD[53]. EGCG administration significantly inhibits β-secretase activity and protects against mitochondrial damage, which is beneficial for alleviating Aβ-induced neurotoxicity[54]. C3G inhibits $A\beta_{40}$ fibrillogenesis, disintegrates preformed fibrils, and reduces amyloid cytotoxicity[55]. Silibinin decreases Aβ deposition and reduces soluble $A\beta_{42}$ levels in the hippocampus by downregulating APP and BACE-1 and upregulating neprilysin in APP/PS1 (presenilin 1) mice[56]. Oleuropein in olive oil has been shown to reduce neuroinflammation by inhibiting the nuclear factor kappa-B pathway, suppressing the activation of NLRP3 inflammasomes

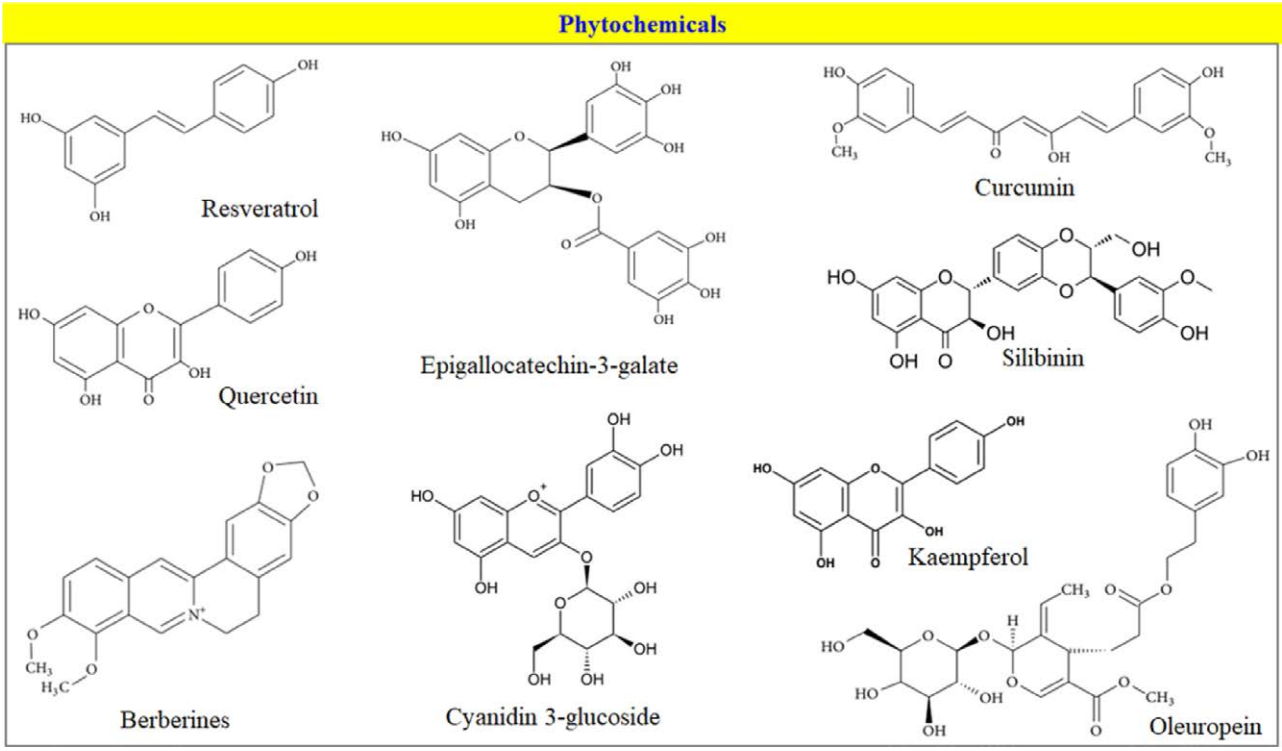

Figure 1. The structure of phytochemicals that could treat neurodegenerative diseases.

and random activation gene expression or high mobility group protein 1 pathways, reducing total Aβ brain levels, and enhancing BBB integrity and function, which collectively improves memory function in AD mice[46]. In PD, Que inhibits α-syn aggregation by covalently binding to α-syn, preventing α-syn fibrillation[38]. Kaempferol induces autophagy by increasing lysosome biogenesis and inducing the expression of transcription factor EB. Additionally, it indirectly reduces the accumulation of α-syn or directly blocks the α-syn amyloid fibril formation to protect against α-syn–related neurotoxicity[57]. Ber increases autophagy-dependent degradation against the accumulation of mHTT[52] and the formation of insoluble TDP-43 aggregates in HD and ALS[58].

Phytochemicals have potential as preventive and therapeutic compounds against NDs; however, poor bioavailability and low accumulated concentrations in brain tissue limit their therapeutic efficacy in clinical trials[59]. Some phytochemical derivatives have been synthesized and modified to produce BBB-permeable compounds; however, phytochemical distribution, metabolism, and bioactivity in the brain may change to some extent. Therefore, designing and developing targeted drug delivery systems capable of crossing the BBB with more stable phytochemicals accumulated in the brain is critical.

EVs for drug delivery

EVs are secreted by various cells and serve different functions. EVs in the blood circulation of the body can cross the BBB and enter the CNS through direct transendothelial or antiaxoplasmic transport[60]. They regulate the development and regeneration of neurons as well as synaptic function[61]. Therefore, we summarize the sources, compositions, and bioactivities of EVs, which were used as drug carriers for NDs.

Source, composition, and biological effects of EVs

EVs are small membranous vesicles that are naturally produced and excreted by numerous cell types. EVs include exosomes (40 nm–120 nm), microvesicles (100 nm–1 μm), apoptotic bodies (50 nm–2 μm), and oncosomes (1 μm–10 μm)[62]. Exosomes are small nano-sized vesicles that have been studied for biomedical applications[63]. Animal-derived exosomes can be harvested from cells or bodily fluids, and plants, like animals, excrete exosome-like nanovesicles containing information molecules[64]. Exosomes contain various biomolecules from donor cells, including lipids, proteins, and nucleic acids. The lipid bilayer of exosomes is enriched with sphingomyelin, gangliosides, and desaturated lipids, but their phosphatidylcholine and diacylglycerol proportions decrease relative to the membranes of their cells of origin[65]. Proteins in exosomes include major histocompatibility complex class II and tetraspanins (CD37, CD53, CD63, CD81, and CD82), endosomal sorting complex required for transport proteins, ALG-2 interacting protein-X, tumor susceptibility gene 101, and heat shock chaperones (Hcs70 and Hsp90)[66]. Nucleic acids in exosomes are predominantly long non-coding RNAs, microRNAs (miRNAs), and messenger RNAs[67].

EVs circulate through all bodily fluids and play a major role in intracellular and intercellular communication because of their ability to transfer proteins and nucleic acids from one cell to another[68]. The biological effects of exosomes mainly depend on the parent cells, such as mesenchymal stem cells (MSCs), immunocytes, neurons, and other cell types (Figure 2), as well as blood, plasma, saliva, milk, and urine[69]. Among them, MSCs are the most expected source of EVs, as they serve as a bridge and link of communication between stem cells and injured cells, promoting the communication of information between cells, regulating immunity, and facilitating self-repair of

Table 1

Major effects of phytochemicals on neurodegenerative disease progression

Phytochemicals	Targeted molecules	Effects	Disease	Reference
Resveratrol	1) SOD, CAT, GSH↑, ROS, MDA↓; 2) ↓NLRP3, TLR4/NF-κB, HMGB1, ↑M2 microglia polarization; 3) ↓mtDNA fragmentation, ↑MMP, ↑mitophagy; 4) ↓BAX/Bcl-2, ↓caspase-3/9, ↓p38, JNK; 5)↓Aβ	1) anti-oxidant; 2) anti-inflammatory; 3) mitochondrial protection; 4) anti-apoptotic; 5) anti-aggregation effect	AD	Yu et al. 2018[29], Ladiwala et al. 2010[30], Karuppagounder et al. 2009[31]
Curcumin	1) ↑GSH ↑NRF2, ↓ROS ↓MDA; 2) ↓TNF-α, IL-1β, IL-1α, ↓TLR4, ↑M2 microglia polarization; 3) ↑MMP ↑ATP, ↑mitophagy; 4) ↓BAX/Bcl-2, Caspase-3/9; 5) ↓Aβ, ↓α-syn	1) anti-oxidant; 2) anti-inflammatory; 3) mitochondrial protection; 4) anti-apoptotic; 5) anti-aggregation effect	AD, PD	Zhang et al. 2017[32], Xia et al. 2016[33], Jiang et al. 2013[34], Sharma et al. 2018[35]
Quercetin	1) ↑GSH, ↓LDH ↓ROS ↓MDA; 2) ↓TLR4, ↓COX-2, ↓IL-1β, IL-6, TNF-α; 3) ↑MMP, ↓ultrastructural alterations; 4) ↓caspase-3, ↓BAX/Bcl-2; 5) ↓ Aβ, α-syn	1) anti-oxidant; 2) anti-inflammatory; 3) mitochondrial protection; 4) anti-apoptotic; 5) anti-aggregation effect	AD, PD	Liu et al. 2019[36], Regitz et al. 2014[37], Zhu et al. 2013[38], El-Horany et al. 2016[39]
Cyanidin-3-O-glucoside (C3G)	1) SOD, CAT, GSH↑, ROS, MDA↓; 2) ↑MMP; 3) ↓COX-2, ↓IL-1β, IL-6, TNF-α; 4) ↓caspase-3, ↓BAX/Bcl-2; 5) ↓Aβ, α-syn	1) anti-oxidant; 2) anti-inflammatory; 3) mitochondrial protection; 4) anti-apoptotic; 5) anti-aggregation effect	AD, PD	Pogacnik et al. 2016[40], Winter et al. 2017[41], Rehman et al. 2017[42]
Epigallocatechin-3-galate (EGCG)	1) SOD, CAT, GSH↑, ROS, MDA↓; 2) ↓BAX/Bcl-2, ↓caspase-3/9; 3) ↓mtDNA fragmentation, ↑MMP, ↑mitophagy; 4) ↓ Aβ, α-syn	1) anti-oxidant; 2) mitochondrial protection 3) anti-apoptotic; 4) anti-aggregation effect	AD, PD	Chen et al. 2018[43], Šneideris et al. 2015[44], Xu et al. 2020[45]
Oleuropein	1) ↓NLRP3, NF-κB, HMGB1; 2) ↓Aβ	1) anti-inflammatory; 2) anti-aggregation effect	AD	Abdallah et al. 2022[46]
Kaempferol	1) ↑SOD ↑GSH ↑NRF2, ↓ROS ↓SDH ↓MDA; 2) ↓NLRP3, ↓TNF-α, IL-1β; 3) ↑MMP ↑ATP ↑mitophagy; 4) ↓caspase-3/9, ↓JNK/p38 MAPK; 5) ↓α-syn	1) anti-oxidant; 2) anti-inflammatory; 3) mitochondrial protection; 4) anti-apoptotic 5) anti-aggregation effect	PD	Filomeni et al. 2012[47], Han et al. 2019[48]
Berberine	1) ↓ROS, ↑NRF2-HO-1; 2) ↓IL-1β, TNF-α; 3) ↑MMP; 4) ↓caspase-3, ↓BAX/Bcl-2; 5) ↓Aβ, APP, p-tau, ↓polyQ-Htt, ↓TDP-43	1) anti-oxidant; 2) anti-inflammatory; 3) mitochondrial protection; 4) anti-apoptotic; 5) anti-aggregation effect	AD, HD, ALS	Chen et al. 2020[49], Huang et al. 2017[50], Jiang et al. 2015[51], Chang et al. 2016[52]

α-syn: Alpha-synuclein; Aβ: Aggregated β-amyloid; AD: Alzheimer disease; ALS: Amyotrophic lateral sclerosis; APP: Amyloid precursor protein; ATP: Adenosine triphosphate; BAX/Bcl-2: B-cell lymphoma protein 2-associated X; GSH: Glutathione; IL-1α: Interleukin-1 alpha; HD: Huntington's disease; IL-1β: Interleukin-1 beta; JNK: c-Jun N-terminal kinases; MDA: Malondialdehyde; MMP: Matrix metalloproteinases; NLRP3: Nucleotide-binding and oligomerization domain-like receptor protein 3; NRF2: Nuclear factor-erythroid 2-p45 derived factor 2; PD: Parkinson disease; ROS: Reactive oxygen species; SDH: Succinate dehydrogenase; SOD: Superoxide dismutase; TNF-α: Tumor necrosis factor alpha.

damaged cells[70]. MSC-derived EVs can easily cross the BBB and directly stimulate neuron regeneration. EVs from neurons[60], astrocytes[71], oligodendrocytes[72], or microglial cells[73] in the CNS are released to transport proteins, siRNA, and miRNA to remove cellular metabolic waste, regulate immune responses, and adjust neuron and glial cell growth, regeneration, and synaptic regulation[74]. Immune cells, such as dendritic cells and macrophages, migrate to pathological areas and regulate the inflammatory response[75]. Breast milk naturally secretes EVs that contain high levels of immune-related miRNAs that can be transferred to infants and exert immunomodulatory effects[76]. Studies have shown that EVs from cow milk have significant anti-inflammatory, tolerogenic, and anti-apoptotic effects in various settings[77]. Plant-derived EVs, mainly isolated from vegetables, fruits, grains, and herbs, have emerged in recent years owing to their ease of acquisition, large production, and non-immunogenicity[78]. In recent decades, several plant-derived EVs have been registered in clinical trials, including grapes, ginger, and aloe-derived exosomes[79]. EVs in various intercellular processes are involved in the pathology of NDs, and their natural origin and bioactive molecules within EVs influence their therapeutic potential in brain diseases (Table 2).

EVs are isolated from these cells using different isolation techniques, including ultracentrifugation, density gradient centrifugation, exosome precipitation, antibody-based immunoaffinity purification, tangential flow filtration, and nano-flow cytometry, to develop their biomedical application[86]. Isolated EVs must be evaluated for cytotoxicity and immunogenicity before being used *in vivo*.

EVs as drug carriers

EVs have many advantages as drug carriers, including innate stability, low immunogenicity, and tissue barrier permeability[87]. First, EVs protect cargo from acidic environments and enzyme degradation because of their double membrane[88]. Nanometer-sized EVs also circulate in the body for a long time to maintain their functions. Owing to their cell origin, exosomes can avoid phagocytosis or degradation by macrophages, thereby avoiding endosomal degradation of drugs. Second, EVs are generally obtained from the patient's recipient cells

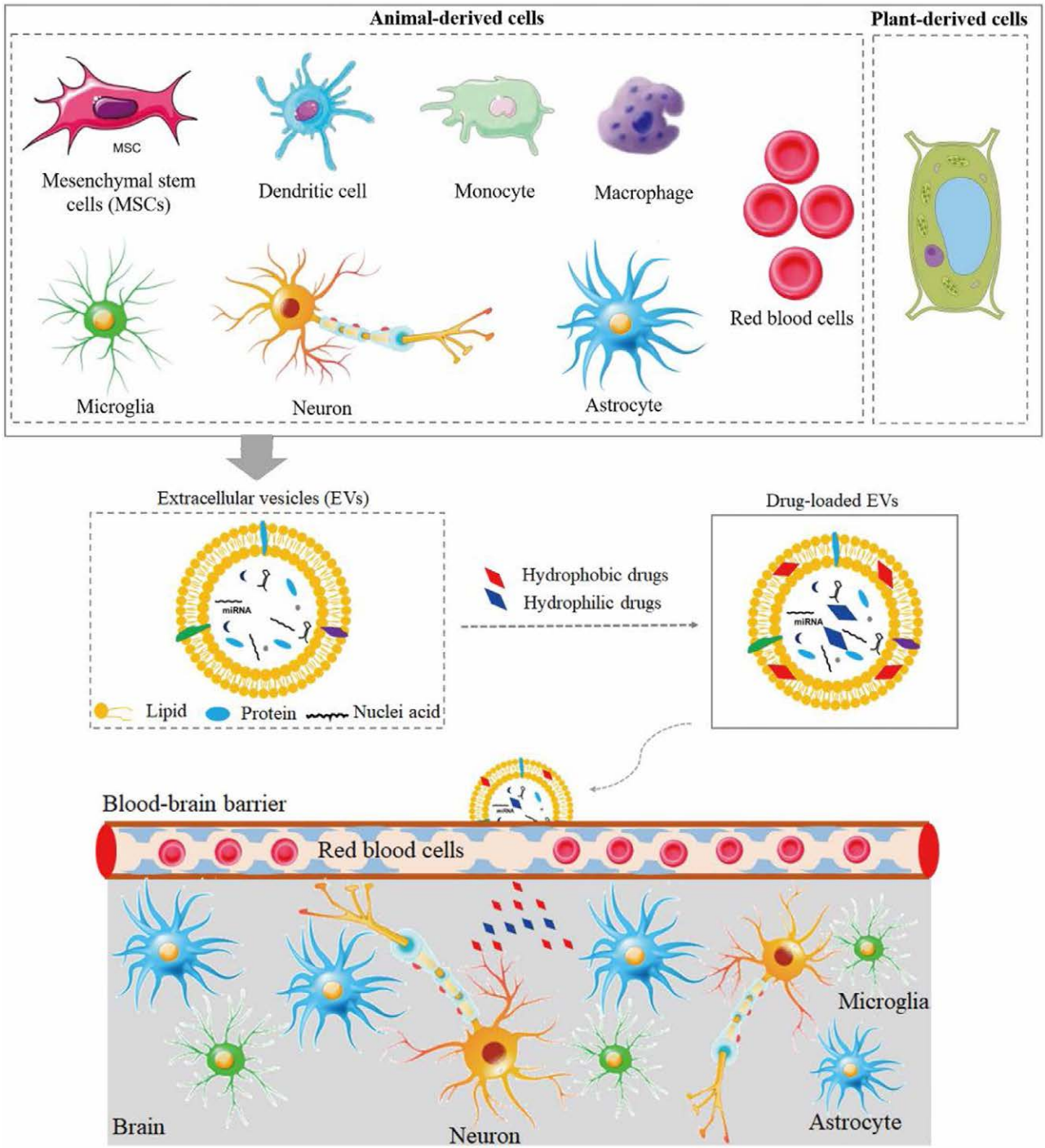

Figure 2. Sources and composition of exosomes, and their potential as drug carriers to cross the blood-brain barrier in the central nervous system.

to eliminate potential immune complications and ensure that EV recognition in clinical trials is similar to that of their recipient cells[89]. Third, EVs could cross tissue barriers, including the intestinal barrier and BBB. Therefore, EVs are excellent drug delivery candidates.

As the BBB is one of the major obstacles in treating NDs, it is important to understand how EVs interact with the BBB. EVs secreted by brain cells can cross the BBB into the bloodstream, and circulating EVs can cross the BBB into the brain[90]. To date, exosomes and receiving cells interact in five ways: recognition by a protein G-coupled receptor on the cell surface, adhesion and fusion to the cell surface, micropinocytosis, non-specific or lipid rafts, and receptor-mediated transcytosis[91]. Exosomes might be degraded by lysosomes or their content released into the cytoplasm through a back fusion event or trafficking from the multivesicular body to the plasma membrane as neo formed intraluminal vesicles in the receiving cell[92].

Efficient drug loading into EVs is critical for successful therapies. There are two methods for encapsulating cargos into EVs: endogenous and exogenous drug-loading methods (Figure 3). In the endogenous drug-loading method, desired cargos are incubated with cells that can produce EVs, relying on the natural mechanisms of EVs to package natural compounds into EVs[93]. EVs possess an aqueous core and

Table 2

Extracellular vesicles derived from different cells for neurodegenerative disease treatment

EV source	Models	Disease	Outcomes	Reference
Mouse bone marrow derived–MSCs	APP/PS1 mice	AD	Aβ-induced iNOS expression suppressed	Wang et al. 2018[80]
Human bone marrow–derived MSCs	6-Hydroxydopamine-induced rat	PD	Dopaminergic neurons increased, recovery of motor performance outcomes	Teixeira et al. 2017[81]
Human adipose–derived stem cells	R6/2 mice-derived neuronal stem cells for HD model	HD	mHTT aggregates decreased, mitochondrial dysfunction and cell apoptosis reduced	Lee et al. 2016[82]
Murine adipose–derived stem cells	SOD1 transgenic mice	ALS	Motor performance improved, glial cells activation decreased	Bonafede et al. 2020[83]
Neuro 2A (N2a) cells	APP mouse	AD	Aβ deposition–mediated synaptotoxicity in the hippocampus reduced	Chang et al. 2013[84]
Astrocytes	Full-length HTT 140Q KI mice	HD	mHTT aggregation-induced cellular toxicity reduced	Hong et al. 2017[85]

Aβ: β-amyloid; AD: Alzheimer disease; ALS: Amyotrophic lateral sclerosis; APP/PS1: Amyloid precursor protein/presenilin 1; HD: Huntington's disease; mHTT: Mutant huntingtin protein; MSCs: Mesenchymal stem cells; PD: Parkinson disease; SOD1: Superoxide dismutase 1.

a lipophilic shell formed by the lipid bilayer, which allows them to store and dissolve hydrophobic and hydrophilic compounds through non-specific binding interactions[94]. This is a relatively simple strategy for constructing drug delivery systems because of the cage-like internal structures of EVs[95]. Drug-loaded EVs are released by heat, hypoxia, and other stimuli[96]. The first step in the exogenous drug-loading method is the isolation of EVs, and the desired cargo is then loaded into the EVs using mechanical approaches. Simple incubation, sonication, electroporation, freeze or thaw cycles, saponin permeabilization, and mechanical extrusion have been employed to encapsulate drugs in exosomes[97]. Exogenous drug-loading techniques differ depending on the solubility of the desired cargo. Hydrophobic compounds are relatively easy to load because they interact by mixing and incubating with the lipid bilayer of the EV membrane *via* hydrophobic interactions. Successful drug loading has been reported for curcumin[98] and doxorubicin[99].

Drug loading of hydrophilic natural compounds can be manipulated by electroporation, ultrasound, freeze or thaw cycles, and saponin permeabilization. Electroporation is usually used to load nucleic acids into EVs and for transfection with commercially available reagents[100]. Ultrasonic treatment increased drug load capacity and supported the release of EVs excreted by macrophages[100]. This strategy requires an isolation method that produces high yields of purified exosomes free of impurities. Moreover, this method may compromise functionality[101] and the physicochemical properties of drug molecules may affect the stability and bioactivity of EVs[94]. Exogenous drug loading was used to co-incubate anthocyanidins with mature bovine

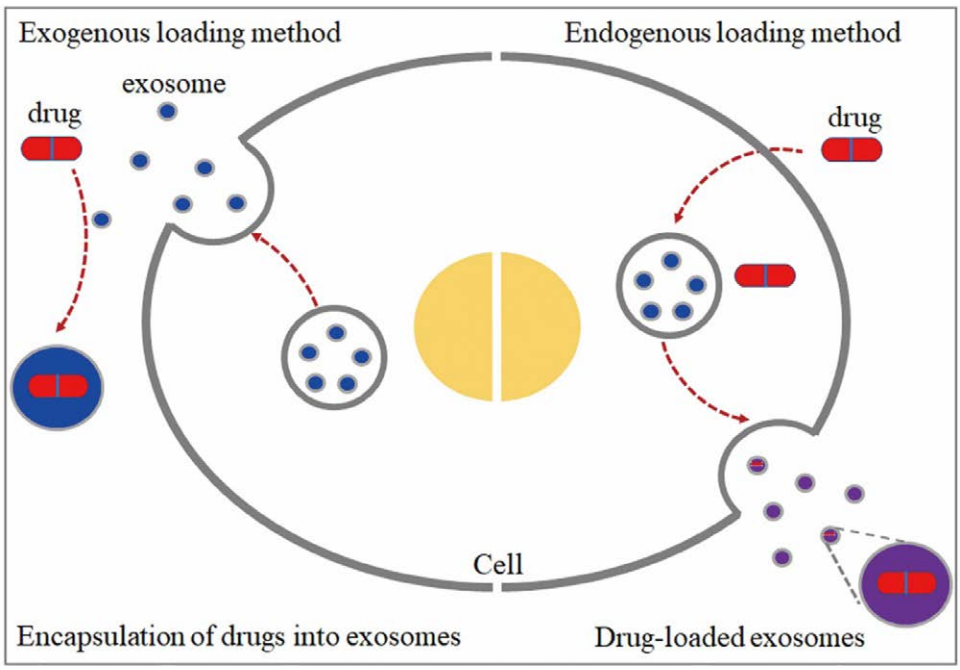

Figure 3. Encapsulation strategies of drugs into exosomes.

milk-derived exosomes (Exo-Anthos), and Exo-Anthos significantly enhanced the drug's oral bioavailability and its bioactivity[102].

Unlike exogenous loading methods, endogenous drug-loading methods do not cause EV denaturation because natural compounds are sorted into EVs based on their natural cell mechanism. However, endogenous loading methods have a much lower drug-loading efficiency than exogenous loading methods. Therefore, loading strategies must be optimized to ensure effective utilization of exosomes as drug delivery systems.

Applications of phytochemical-loaded EVs for ND therapy

Phytochemicals have huge potential as preventive or therapeutic agents; however, their clinical application has several limitations. First, the solubility of phytochemicals is essential for absorption and eliciting biologically efficient blood levels. The absorption of phytochemicals is lower in powder form than in soluble form[103]. Following oral administration of a 500 mg tablet, the plasma level of resveratrol is of nM magnitude, and the plasma level of its metabolites can reach μM magnitude[104]. However, resveratrol plasma levels in beverages (red wine) can reach higher concentrations within several hours[105]. Approximately 75%[106] or 60% to 66%[107] of the dose is absorbed following the oral administration of resveratrol or curcumin, respectively, but these absorptions are insufficient as therapeutic agents. Second, the administration methods affect the concentration accumulation of phytochemicals in brain tissue. Oral delivery of curcumin is less effective because of its low solubility in water, poor absorption, and rapid biotransformation, resulting in lower systemic bioavailability[108]. Notably, tail vein injection, or more precisely, intracerebral injection through a cannula, prevents brain tumor formation in mice[109]. Third, most phytochemicals exhibit low bioavailability owing to their short biological half-life, rapid metabolism, and clearance. Based on previous studies, the oral bioavailability of resveratrol, curcumin, and C3G was less than 1%[110–112]. Their low bioavailability prevents drug accumulation at the concentrations necessary for successful therapy in target tissues. Fourth, the concentrations of phytochemicals and their metabolites did not reach the concentrations expected to achieve *in vivo* biological effects. A previous study demonstrated that C3G levels in the brain of rats intravenously administered a bolus of 668 nmol C3G were only nM[113]. Numerous studies *in vitro* and *in vivo* have demonstrated that the concentration accumulated in the target tissue should reach a μM magnitude to interact with molecular targets and influence the related pathways.

New strategies are required because the success of an ND therapy depends greatly on the higher solubility, absorption, bioavailability, and accumulated concentration of phytochemicals in the brain. Considering the superiority of EVs as natural drug-loading tools that offer various advantages, harnessing their beneficial properties to overcome the limitations of phytochemicals is a promising prospect. In this section, we review the most recent studies on the applications of phytochemical-loaded EVs in ND therapy (Table 3).

Curcumin

Curcumin is a hydrophobic molecule with a logP value of approximately 3.0, and a molecular weight of 368.38 Da. It has a simple structure with two phenolic functional groups connected by a conjugated b-diketone system[120]. Only molecules with high lipophilicity and low molecular weight can cross the BBB. Unfortunately, non-specific absorption and poor bioavailability of curcumin in the brain were inevitable. Wang et al.[114] proposed that exosomes derived from curcumin-primed macrophage RAW264.7 cells (Exo-Cur) should be harvested using

Table 3

Phytochemical-loaded extracellular vesicles for neurodegenerative disease therapy

Phytochemicals	EV source	Models	Administration	Outcomes	Reference
Curcumin	RAW264.7 cells	Okadaic acid–induced AD rats	Peritoneal injection	BBB penetration of drug increase; Tau phosphorylation inhibited; learning and memory ability improved	Wang et al. 2019[114]
Resveratrol	Microglia	Spinal cord injury rat model	Intraperitoneal injection	The neuronal survival rate and autophagy rate increased; the locomotor function improved	Fan et al. 2020[115]
Silibinin	RAW264.7 cells	$A\beta_{42}$-induced AD mouse model	Injected daily into mice *via* the tail vein	Aβ aggregation reduced; astrocyte inflammation-mediated neuronal damage alleviated; cognitive deficits ameliorated	Huo 2021[116]
Curcumin	MSCs from mice bone marrow	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine–induced PD mice	Nasal administration	α-Synuclein aggregates reduced, the neuron function recovered, neuroinflammation alleviated; the movement and coordination ability of mice improved	Peng et al. 2022[117]
Quercetin	Plasma exosomes	Okadaic acid–induced AD rats	Intravenous injection	Tau protein aggregation inhibited	Qi et al. 2020[118]
Berberine	M2-type primary macrophages	Spinal cord injury rat model	Tail vein injection	Anti-inflammatory and anti-apoptotic effect enhanced; the motor function of mice improved	Gao et al. 2021[119]

AD: Alzheimer disease; BBB: Blood-brain barrier; MSCs: Mesenchymal stem cells; PD: Parkinson disease.

an endogenous drug-loading method. The encapsulation efficiency and loading capacity of curcumin in exosomes were 84.8% and 15.1%, respectively. Therefore, exosomes improved the solubility and bioavailability of curcumin and targeted brain tissues with highly effective BBB-crossing *via* receptor-mediated transcytosis. Moreover, Exo-Cur inhibited tau phosphorylation by activating the AKT/glycogen synthase kinase-3β pathway and the recovery of neuronal function in AD therapy[114]. Exogenous drug-loading methods have also been used to successfully load curcumin into many types of cell-derived exosomes, such as curcumin-loaded embryonic stem cell exosomes (MESC-Exo-Cur). Administration of MESC-Exo-Cur *via* the nasal route in ischemia-injured mice improved the neurological score[121]. Peng et al.[117] developed another complex strategy in which they embedded curcumin micelles into functionalized MSC-derived exosomes to create a self-oriented nanocarrier, PR-EXO/PP@Cur. The loading capacity of curcumin in the nanocarrier was 75.53%. It can resist the clearance of the nasal mucosa across multiple membrane barriers and accurately identify target neuronal cells after intranasal administration. Curcumin accumulation at the action site effectively reduces α-syn aggregates, promotes neuron function recovery, alleviates neuroinflammation, and improves movement and coordination ability in PD model mice[117].

Silibinin

Silibinin, a flavonoid compound, has poor delivery across the BBB, with a log*P* value of 0.86 and a molecular weight of 482.44 Da. In addition, the low bioavailability of silibinin contributes to its poor clinical efficacy in NDs. Huo et al.[116] isolated exosome-silibinin (Exo-Slb) by co-incubating silibinin with RAW264.7 cells, an endogenous drug-loading method. The encapsulation efficiency and loading capacity of silibinin in exosomes were 41.8% and 21.2%, respectively. Exo-Slb can improve the brain-targeting ability of silibinin. After entering the brain, Exo-Slb selectively interacts with Aβ monomers to reduce aggregation. Simultaneously, Exo-Slb is internalized in astrocytes to inhibit their activation and alleviate astrocyte inflammation-mediated neuronal damage, thus ameliorating cognitive deficits in AD mice[116].

Resveratrol

Resveratrol-primed exosomes (Exo-Res) secreted by primary microglia from rats were collected using an endogenous drug-loading method, which solved the problems associated with drug solubility and bioavailability of resveratrol. Additionally, Exo-Res displayed greater enhancement of neuronal survival and increased autophagy rates, while reducing apoptosis levels than free resveratrol in spinal cord injury (SCI) model rats[115].

Que

Que was encapsulated in blood plasma-derived exosomes using an exogenous drug-loading method. Que had an encapsulation efficiency and drug-loading capacity of 30.00 ± 8.30% and 17.30 ± 6.34%, respectively. The bioavailability of Que improved as the area under the plasma concentration–time curve from zero time to infinity (AUC_{0-t}) of Que in exosome-que (Exo-Que) increased to 7.5-fold when compared to free Que. Exo-Que significantly improved the brain-targeting and bioavailability of Que. Exo-Que inhibited cyclin-dependent kinase 5-mediated tau phosphorylation and reduced the formation of insoluble neurofibrillary tangles, improving cognitive function in AD mice[118].

Ber

Ber was directly mixed with exosomes derived from M2-type primary macrophages in mice using an exogenous drug-loading method. The Ber content in exosome-Ber (Exo-Ber) was 17.13 ± 1.64% after ultrasonication. Exo-Ber was slowly released *in vitro*. At 48 h, the cumulative release reached 71.44 ± 2.86%. The AUC_{0-inf} of Exo-Ber was three times higher than that of the free Ber. Exo-Ber can be efficiently targeted to deliver drugs to the injured spinal cord owing to the natural advantage of exosomes across the BBB. Exo-Ber had anti-inflammatory and anti-apoptotic effects by inducing macrophage or microglial polarization from the M1 phenotype to the M2 phenotype. Moreover, Exo-Ber treatment significantly improved motor function in SCI mice[119].

Conclusions and perspectives

NDs are characterized by nervous system damage, mainly caused by misfolded protein aggregation, inflammation, oxidative stress, mitochondrial dysfunction, and genetic mutations in the brain. BBB significantly limits the therapeutic efficacy of clinically approved drugs. EVs are likely to solve this problem because they can pass through the BBB and deliver drugs to the brain. Phytochemicals have proven to be preventive and therapeutic compounds for NDs. However, several unresolved issues, such as instability, solubility, low bioavailability, inability to cross the BBB, and lack of selectivity for brain lesion sites, limit the use of phytochemicals to treat brain disease. Nano-sized EVs have a tendency to cross the BBB, attach to cellular membranes through receptor–ligand interactions, release functional cargos, and avoid the endosomal pathway and lysosomal degradation. In particular, exosomes are used to encapsulate phytochemicals to enhance their accumulation in the brain. Exosome products, such as ExoPr0, derived from cyclophosphamide neural stem cells[122] are approaching clinical trials for the treatment of NDs. In this review, we elucidated the emerging applications of phytochemical-loaded EVs in ND treatment. A brief description of the encapsulation strategies, mechanisms of action, and therapeutic outcomes in AD, PD, and other brain disorders was provided.

Notwithstanding, several challenges still exist before phytochemical-loaded EVs can be used in clinical applications, including low reproducibility, limited drug-loading capacity, and safety concerns. First, the source of EVs is critical, and standardization of separation techniques suitable for mass production and high purification is required. Stem cells are an excellent choice for the production of EVs. Second, the

composition of EVs derived from the different types of parent cells varies significantly. Biomimetics that combine the versatility of synthetic cores with the outer shell of EVs for their functional properties to produce therapeutic nanoparticles have been developed in recent years to solve this issue. However, this approach raises new concerns on how to maintain the structure of EVs during cargo loading, as EV membrane integrity is crucial for their bioactivities. Third, optimal techniques for high drug-loading capacity into EVs are still lacking because EVs have low loading capacity compared to synthetic nanoparticles, especially for the loading of hydrophilic phytochemicals. Fourth, the stability, storage temperature, or time of phytochemical-loaded EVs before administration are challenging. Milk-derived exosomes are stable during harsh processing and long-term storage; however, the stability of plant-derived EVs during processing is unknown. Fifth, the release rate and pharmacokinetics of phytochemicals should be well controlled to ensure the therapeutic efficacy of phytochemical-loaded EVs in the brain. Therefore, the application of phytochemical-loaded EVs to combat NDs still has a long way to go as an alternative therapeutic approach.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

Zhenzhu Zhu conceived, designed, and drafted this review; Liuyue Liao collected the data and references; Hongzhi Qiao revised the manuscript. All the authors have read and approved the final manuscript.

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