REVIEW
Neuroprotective Natural Products for the Treatment of Parkinson’s Disease by Targeting the Autophagy–Lysosome Pathway: A Systematic Review

Zi-Ying Wang,1,2 Jing-Yi Liu,1,2 Chuan-Bin Yang,1,2 Sandeep Malampati,1,2 Ying-Yu Huang,1,2 Mei-Xiang Li,1,2 Min Li1* and Ju-Xian Song1*
1School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, SAR, China
2Mr. and Mrs. Ko Chi-Ming Centre for Parkinson’s Disease Research, Hong Kong Baptist University, Kowloon Tong, Hong Kong, SAR, China

The autophagy–lysosome pathway (ALP) is a primary means by which damaged organelles and long-lived proteins are removed from cells and their components recycled. Impairment of the ALP has been found to be linked to the pathogenesis of Parkinson’s disease (PD), a chronic neurodegenerative disorder characterized by the accumulation of protein aggregates and loss of dopaminergic neurons in the midbrain. In recent years, some active compounds derived from plants have been found to regulate the ALP and to exert neuroprotective effects in experimental models of PD, raising the possibility that autophagy enhancement may be an effective therapeutic strategy in PD treatment. In this review, we summarize recent findings of natural products that enhance ALP and thereby protect against PD. Research articles were retrieved from PubMed using relevant keywords in combination. Papers related to the topic were identified, and then the reliability of the experiments was assessed in terms of methodology. The results suggest that targeting the ALP with natural products is a promising strategy for PD treatment. However, risk of bias exists in some studies due to the defective methodology. Rigorous experimental design following the guidelines of autophagy assays, molecular target identification and in vivo efficacy evaluation is critical for the development of ALP enhancers for PD treatment in future studies. Copyright © 2017 John Wiley & Sons, Ltd.

Keywords: Parkinson’s disease; autophagy; neuroprotection; natural products.

INTRODUCTION

Autophagy, or the autophagy–lysosomal pathway (ALP), is an intracellular process that degrades and recycles cytosolic components, including misfolded proteins and damaged organelles (Choi et al., 2013). There are three types of autophagy, namely macroautophagy, chaperone-mediated autophagy (CMA) and microautophagy. Macroautophagy is the most common pathway, and, for the rest of this review, the word ‘autophagy’ will refer to macroautophagy. Autophagy can be stimulated by different pathological and physiological conditions (Ravikumar et al., 2010). For example, during starvation, autophagy degrades proteins, carbohydrates and lipids to compensate for nutrient deprivation. In most cells, autophagy occurs at a basal rate in order to maintain cytoplasmic homeostasis (Rubinsztein et al., 2012), by degrading dysfunctional organelles and toxic protein aggregates (Yang and Klonosky, 2010a, 2010b). The initiation of autophagy includes the formation of a double-membrane vesicle termed autophagosome, which, in mammalian cells, forms randomly in multiple locations (Mizushima and Levine, 2010). More than 15 autophagy-related protein (ATGs) have been identified as being involved in the formation of autophagosomes (Rubinsztein et al., 2011). Golgi body complex and the endoplasmic reticulum (ER) are potential membrane sources for the generation of autophagosomes (Ohashi and Munro, 2010). Once the autophagosomes are formed, they fuse with lysosomes to degrade the cellular contents (Mizushima et al., 2011).
Parkinson’s disease (PD) is a progressive neurodegenerative movement disorder pathologically characterized by the loss of dopaminergic neurons in the midbrain and accumulation of abnormal α-synuclein aggregates termed Lewy bodies. Previous studies have revealed the key roles of oxidative stress in PD and the application of natural antioxidants as possible neuroprotective therapies (Sarrafchi et al., 2016). In recent years, increasing evidence has shown that ALP impairment plays important roles in the pathogenesis and development of PD (Gan-Or et al., 2015; Pan et al., 2008). Both genetic factors and environmental neurotoxins have been shown to impair the ALP and thereby lead to neurodegeneration in PD (Gan-Or et al., 2015; Pan et al., 2008). Targeting ALP represents a promising strategy for disease-modifying treatment of PD. Some small molecules have been identified as autophagy inducers or ALP enhancers; these molecules can degrade the toxic protein aggregates and attenuate neurodegeneration in experimental PD models. Among the ALP modulators identified, natural products from traditional herbal medicines are receiving increasing attention partly because of their long-term usage in folk medicine which suggests efficacy as well as low toxicity (Sewell and Rafieian-Kopaei, 2014; Nasri and Shirzad, 2013). In this review, we summarize the ALP enhancers discovered from natural products and their effects in PD models.

MATERIALS AND METHODS


Study selection. After reading titles and abstracts, 21 research articles describing the protective effects of natural products in in vitro and/or in vivo models of PD by regulating ALP were selected for further evaluation.

Data extraction and quality assessment. The methodology and results of each article were critically analyzed according to the Autophagy guidelines (Klionsky et al., 2016), and the risk of bias in the experimental design was estimated. The studies which did not fulfill the general protocols of autophagy assays were excluded. Data were extracted from the final 14 papers for this review (Fig. 1). Relevant papers were also included in our review after surveying the references from the selected articles.

RESULTS

The regulation of autophagy

In eukaryotic system, the activity of autophagy is highly regulated by a wide variety of factors, including growth factors, glucose, amino acids and energy status. These factors are the signals integrated by the kinase activity of mammalian target of rapamycin (mTOR) (Kim et al., 2002). Mammalian target of rapamycin is a key homeostatic regulator for autophagy by forming two different complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Zheng et al., 2016). Of these two, mTORC1 is an essential controller that regulates many cellular processes, including protein synthesis and autophagy. Exposure to rapamycin or starvation induces autophagy by inhibiting mTORC1 (Cai and Yan, 2013). In addition, several different non-ATG proteins are involved in regulating autophagy activity, e.g., AMPK, AKT and vacuolar protein-sorting protein 34 (VPS34), which is a member of class III PI3K (Yang and Klionsky, 2010a, 2010b). The main mechanisms of autophagy regulation are summarized in Fig. 2.

PI3K/AKT/mTOR pathway. Various signaling pathways control autophagic activity in mammalian cells. Studies have revealed that the autophagy inducer rapamycin does not inhibit mTORC1 completely, whereas two other small-molecule mTOR inhibitors, PP242 and Torin1, can both inhibit mTORC1 and mTORC2 (Thoreen and Sabatini, 2009). Besides, mTORC1 can be regulated by the upstream class I phosphoinositide 3-kinase (PI3K)/AKT pathway. Phosphoinositide 3-kinase activity is promoted by growth factors such as insulin-like growth factor-1 (IGF1) and phosphorylation of AKT (Heras-Sandoval et al., 2014). Consecutively, AKT promotes mTOR activity by phosphorylating tuberous sclerosis complex 2 (TSC2) and binding to 14-3-3 protein. The phosphorylation of TSC2 stimulates...
Ras homolog enriched in brain (Rheb) and promotes mTOR activation (Dunlop and Tee, 2014). The downstream kinases of mTOR include p70 ribosomal S6 kinase (p70S6K) and eukaryotic initiation factor 4E binding protein 1 (4EBP1), which are related to cell growth and translational machinery (Klionsky and Emr, 2000).

**AMPK/ULK1/mTOR pathway.** Besides, autophagy can be promoted by AMP-activated protein kinase (AMPK), which can be activated by the increase of AMP/ATP ratio and is an energy sensor that modulates the cellular metabolism (Meley et al., 2006). It is reported that AMPK inhibits mTORC1 by phosphorylating and activating the ULK1 complex (Kim et al., 2011). Conversely, under nutrient sufficiency conditions, mTORC1 disrupts the ULK1–AMPK interaction by the direct phosphorylation of ULK1.

**Bcl-2/Beclin-1 pathway.** Beclin-1, the Bcl-2 homology (BH3)-only protein, is an essential initiator for autophagy (Pattinigre et al., 2005). The function of Beclin-1 is to recruit the main autophagic proteins that are required for building pre-autophagosomal structures (Erlich et al., 2007). Apart from that, Beclin-1 is the central determining sensor for cells to undergo apoptosis or autophagy. Beclin-1 has been reported to interact with anti-apoptotic Bcl-2 family members (Liang et al., 1998). This interaction inhibits the congregation of Beclin-1 and initiates the formation of pro-autophagosome, thereby inhibiting autophagy (Marquez and Xu, 2012).

**Transcription factor EB (TFEB).** The basic helix–loop–helix leucine zipper transcription factor EB (TFEB) is a new potential target for drugs to regulate autophagy. Basically, TFEB controls autophagy-related gene expression in different stages of autophagy, including the formation of autophagosomes, the fusion of autophagosomes as well as the degradation (Settembre et al., 2011). In normal condition, TFEB exists in the cytoplasm, and starvation induces its translocation into the nucleus. Overexpression of TFEB has been shown to rescue midbrain dopamine neurons from α-synuclein toxicity, indicating that TFEB may play an essential role in PD pathology (Decressac et al., 2013). Activation of TFEB nuclear translocation may serve as a strategy to induce autophagy. It is reported that mTORC1 induces autophagy by regulating TFEB nuclear location and TFEB phosphorylated in Ser211 in an mTORC1-dependent manner. The mTOR inhibitors rapamycin and torin1 have also been reported to activate TFEB by promoting TFEB nuclear translocation (Rocznia-Ferguson et al., 2012; Martina et al., 2012). On the other hand, some small molecules may directly target TFEB to enhance autophagy. For example, flubendazole has been shown to activate the autophagosomal system via the TFEB pathway, which is not dependent on the mTOR pathway (Chauhan et al., 2015).

**Roles of the autophagy–lysosomal pathway in Parkinson’s disease**

The pathological features of PD are the presence of Lewy bodies, which are mainly composed of insoluble fibrillar α-synuclein. Point mutations (A30P, A53T and E46K) in α-synuclein cause familiar PD (Goedert, 2001). Accumulation of abnormal and misfolded α-synuclein protein eventually leads to cell death. In mammalian cells, there are two important pathways to degrade aggregated proteins including α-synuclein; these are the ubiquitin–proteasome system (UPS) and the autophagy–lysosomal pathway (ALP) (Pan et al., 2008; Goedert, 2001). The UPS is the main pathway that controls the degradation of soluble intracellular proteins.
Some studies have demonstrated a convincing link between proteasome dysfunction and PD. In vivo, the exposure to toxins like rotenone or MPTP was found to induce proteasome inhibition and cause neuron loss in the SN (Fornai et al., 2005). It is reported that acute UPS dysfunction can induce and upregulate autophagic flux in different PD models (Ding et al., 2007). The evidence that ALP is involved in PD comes from research that found abundant dysfunctional autophagosomes in PD patients’ brains and that showed the accumulation of α-synuclein could inhibit autophagic activity (Banerjee et al., 2010; Winslow et al., 2010). The unfolded and soluble α-synuclein is degraded by the UPS pathway. The oligomer species of α-synuclein show a much higher dependency on autophagic degradation than nonaggregate-prone species. The reason for this may be that aggregated α-synuclein is unable to access the proteasome’s narrow entrance such that autophagy becomes the default pathway for degradation (Dauer and Przedborski, 2003). On the other hand, the induction of autophagy can increase the clearance of α-synuclein and reduce its toxicity, as reported in in vitro cell models, in Drosophila, as well as in in vivo animal models of PD. Indeed, autophagy plays a vital role in the clearance of α-synuclein even while it is not crucially involved in the turnover of endogenous levels of α-synuclein. In normal cells, the degradation of α-synuclein relies on the CMA and UPS pathways. In the early stage of PD, accumulating α-synuclein unbalances the internal environment; but this may trigger autophagy to clear misfolded protein and return balance to the system. In the late stage of the disease, uncontrolled accumulating α-synuclein impairs all three degradation pathways thereby promoting α-synuclein secretion and contributing to a spread of the pathology. This sequence raises the possibility that enhancing autophagy could serve as a therapeutic strategy in PD treatment (Zirin and Perrimon, 2010; Auluck and Bonini, 2002).

**Autophagy–lysosome pathway enhancers from natural products for the treatment of Parkinson’s disease**

Current pharmacological treatments for PD are symptomatic and associated with various side effects. Accumulating evidence has revealed the anti-ageing and neuroprotective effects of natural products from traditional medicines, which may provide alternative therapies for neurodegenerative disorders including PD (Shen et al., 2016; Kim et al., 2016; Li et al., 2016; Islam et al., 2016; Solanki et al., 2016; Sun et al., 2015). Because targeting ALP has recently proposed as a promising strategy for PD and other neurodegenerative diseases (Menzies et al., 2017), the protective effects of genetically or pharmacologically activated ALP have been tested in experimental models of PD in recent years. Among these small molecule activators of ALP, natural compounds from neuroprotective herbal medicines are receiving increasing attention. More and more novel ALP enhancers have been identified, which show neuroprotective effects in cell and animal models of PD. These natural ALP modulators may serve as leading compounds for new drug development for the treatment of PD. We summarize the main ALP modulators (Fig. 3) identified and discuss their potential application for PD treatment.

**Alkaloids**

**Oxindole alkaloids from Uncaria rhynchophylla (Miq.) Jacks (Gouteng).** Uncaria rhynchophylla is routinely used in traditional Chinese medicine formulas for the treatment of symptoms relevant to Parkinson’s disease

![Figure 3. The structure of natural ALP enhancers studied in experimental PD models.](image-url)
(Kum et al., 2011). We identified several neuronal autophagy inducers from oxindole alkaloids isolated from U. rhynchophylla such as corynoxine B (Cory B) (Lu et al., 2012; Song et al., 2014) and corynoxine (Cory) (Chen et al., 2014). We found that Cory B induces autophagy in neuronal cells in an mTOR-independent but Beclin-1-dependent manner (Lu et al., 2012). Further investigation has shown that Cory B blocks SNCA–HMGB1 interaction and increases HMGB1–Beclin-1 interaction to rescue the impaired autophagy induced by α-synuclein overexpression (Song et al., 2014). We also proved that Cory B promotes the clearance of both wide-type and A53T mutant α-synuclein in neuronal cells and in Drosophila (Lu et al., 2012). Although Cory is an enantiomer of Cory B, it induces autophagy in a mTOR-dependent manner and promotes the clearance of WT and mutant α-synuclein (Chen et al., 2014). Furthermore, we demonstrated that Tianma Gouteng Yin (TGY), a traditional Chinese medicine (TCM) decoction mainly composed of Gouteng and Tianma (Gastrodia elata Blume), exerts neuroprotective effects in animal and cellular models of PD (Liu et al., 2015). The autophagy enhancing compounds in the decoction may partially contribute to the observed neuroprotective effects.

Conophylline

Conophylline is a Vinca alkaloid isolated from the tropical plant Tavertaemontana divaricate and can also be isolated from leaves of Ervatamia microphylla. Conophylline was found to induce autophagy in non-neuronal and neuronal cell lines in an mTOR-independent manner (Sasazawa et al., 2015). By promoting autophagic flux, conophylline promoted the degradation of α-synuclein aggregates and reduced cell death induced by the neurotoxin MPP+. (Sasazawa et al., 2015).

Polyphenols

Curcumin and its derivatives. Curcumin is a polyphenol and an active component of turmeric (Curcuma longa) with various pharmacological effects (Kunnammakara et al., 2016). Curcumin has been reported to be neuroprotective in experimental models of PD through multiple mechanisms, such as preventing oxidative stress and inflammation and inhibiting α-synuclein aggregation and fibrillation (Ji and Shen, 2014). Because the autophagy-inducing activity of curcumin in cancer cells has been well studied (Shinojima et al., 2007), whether autophagy plays a role in the neuroprotective effects of curcumin in PD has been investigated. Curcumin protects against A53T α-synuclein-induced toxicity in vitro (Liu et al., 2011). In SH-SY5Y cells overexpressing A53T α-synuclein, curcumin restored autophagy by inhibiting the mTOR pathway to degrade the accumulated A53T α-synuclein (Jiang et al., 2013). These studies indicate curcumin reduces neurotoxicity by enhancing autophagy-mediated degradation of α-synuclein.

However, the poor absorption and low bioavailability of curcumin restrict its clinical application. To improve the bioavailability and potency, we and others have synthesized a number of curcumin derivatives to screen potent ALP enhancers and neuroprotective reagents. We identified novel mTOR-dependent and -independent autophagy enhancers from monocarbonyl analogs of curcumin (Song et al., 2016). Importantly, we identified a potent TFEB activator termed C1 which directly binds to and activates TFEB without inhibiting mTOR activity (Song et al., 2016). Compound C1 promotes ALP and degrades α-synuclein in vitro and in vivo (Song et al., 2016) and therefore could be used as a leading compound for further PD drug development.

Resveratrol

Resveratrol (3,5,4’-trans-trihydroxystilbene) is a polyphenolic stilbene found in grapes and red wine. Emerging evidence has shown that resveratrol has neuroprotective effects for PD in different in vitro and in vivo models. Resveratrol ameliorates both motor deficits and pathological changes in MPTP-treated mice and promotes autophagic degradation of α-synuclein (Guo et al., 2016). Meanwhile, in a rotenone-induced SH-SYSY cell PD model, resveratrol was found to rescue rotenone-induced apoptosis through HO-1-dependent autophagy (Lin et al., 2014). Mechanistically, resveratrol activates AMPK and sirtuin 1 (SIRT1) to enhance autophagy (Ferretta et al., 2014; Wu et al., 2011).

Amurensin G

Amurensin G is a resveratrol tetramer isolated from the roots of Viitis amurensis. Recently, it has been reported that Amurensin G could attenuate cellular toxicity in a PD model by induction of autophagy. Amurensin G treatment inhibits rotenone-induced apoptosis and decreases α-synuclein in SH-SYSY cells (Ryu et al., 2013). Knockdown of Beclin-1 abolishes the effect of amurensin G, which may indicate that amurensin G induces autophagy through a Beclin-1-dependent pathway (Ryu et al., 2013).

Disaccharides

Trehalose. Trehalose is a natural α-linked disaccharide that can be synthesized by many fungi and plants. Trehalose has been well demonstrated to enhance clearance of aggregate-prone protein-like α-synuclein and mutant huntingtin by activating mTOR-independent autophagy (Sarkar et al., 2007). The neuroprotective effect of trehalose has been examined in vivo and in vitro PD models. In stable inducible PC12 cell lines that overexpress α-synuclein, treatment with trehalose significantly promoted the degradation of A30P and A53T mutants of α-synuclein (Lan et al., 2012). Trehalose also showed beneficial effects on the clearance of α-synuclein in an AVV1/2 A53T α-synuclein rat model of PD (He et al., 2016). Further studies with a Lewy Body model mice revealed that trehalose could increase levels of several chaperone molecules, such as HSP90 and SigmaR1 (Tanji et al., 2015). Trehalose can activate TFEB to enhance ALP activity (Dehay et al., 2010). However, the underlying mechanism of how trehalose induces autophagy is still unclear.
Table 1. Neuroprotective effects of natural products in experimental Parkinson’s disease by regulating autophagy-lysosome pathway

<table>
<thead>
<tr>
<th>Compound</th>
<th>Original plants</th>
<th>In vitro/in vivo models</th>
<th>Effective dose</th>
<th>Main outcomes</th>
<th>Molecular mechanisms</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynoxine</td>
<td>Uncaria rhynchophylla</td>
<td>N2a, SH-SY5Y cells; Drosophila expressing GFP-Ag8a; PC12 cells overexpressing α-syn</td>
<td>6.25–25 μM</td>
<td>↑ Autophagy flux, ↑ Autophagy flux</td>
<td>mTOR dependent</td>
<td>(Chen et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>(Miq.) Jacks</td>
<td>(WT and A53T)</td>
<td>10–100 μM</td>
<td>↓ α-syn aggregates</td>
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<td></td>
<td></td>
<td>N2a cells overexpressing α-syn (WT and A53T)</td>
<td>25 μM</td>
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<tr>
<td></td>
<td></td>
<td>PC12 cells overexpressing α-syn (WT and A53T)</td>
<td>25 μM</td>
<td></td>
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<tr>
<td>Corynoxine B</td>
<td>Uncaria rhynchophylla</td>
<td>N2a, PC12, SH-SY5Y cells, primary neurons</td>
<td>12.5–50 μM</td>
<td>↑ Autophagy flux</td>
<td>Beclin-1 dependent</td>
<td>(Lu et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>(Miq.) Jacks</td>
<td>N2a cells overexpressing α-syn (WT, A53T and A30P)</td>
<td>25 μM</td>
<td>↓ α-syn oligomers and aggregates</td>
<td>Beclin-1 dependent</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>PC12 cells overexpressing α-syn (WT and A53T)</td>
<td>25 μM</td>
<td>restores autophagy impaired by α-syn</td>
<td>↑ HMGB1 cytosolic translocation; ↓ HMGB1-α-syn interaction</td>
<td>(Song et al., 2014)</td>
</tr>
<tr>
<td>Conophylline</td>
<td>Tabernaemontana divaricata</td>
<td>HeLa and PC12 cells treated with MPP +</td>
<td>100 ng/mL</td>
<td>↑ Autophagy flux, ↑ cell viability, ↓ α-syn aggregates</td>
<td>mTOR independent</td>
<td>(Sasazawa et al., 2015)</td>
</tr>
<tr>
<td>Polyphenol-enriched fraction</td>
<td>Corema album</td>
<td>Yeast BY4741 cells, H4 neuroglioma cells</td>
<td>30 μg/mL</td>
<td>↑ Autophagy flux, ↓ α-syn aggregates and toxicity</td>
<td>Unclear</td>
<td>(Macedo et al., 2015)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Curcuma longa</td>
<td>SH-SY5Y cells expressing α-syn (WT and A53T)</td>
<td>6 μM</td>
<td>↑ Autophagy flux, ↓ α-syn</td>
<td>mTOR dependent</td>
<td>(Jiang et al., 2013)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Fallopia japonica</td>
<td>SH-SY5Y cells treated with rotenone</td>
<td>12.5–50 μM</td>
<td>↑ Autophagy flux, ↓ rotenone toxicity</td>
<td>AMPK/SIRT1 activation</td>
<td>(Wu et al., 2011)</td>
</tr>
<tr>
<td>Amuresin G</td>
<td>Vitis amurensis</td>
<td>SH-SY5Y cells treated with rotenone</td>
<td>5–10 μM</td>
<td>↓ rotenone-induced apoptosis, ↓ α-syn</td>
<td>Beclin-1 dependent</td>
<td>(Ryu et al., 2013)</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>Various plants</td>
<td>SH-SY5Y cells treated with rotenone</td>
<td>30 μM</td>
<td>↑ Autophagy flux, ↓ rotenone toxicity</td>
<td>Unclear</td>
<td>(Filomeni et al., 2012)</td>
</tr>
<tr>
<td>Celastrol</td>
<td>Tripterygium wilfordii</td>
<td>SH-SY5Y cells treated with rotenone</td>
<td>0.4–1 μM</td>
<td>↓ rotenone toxicity, ↓ α-syn</td>
<td>Unclear</td>
<td>(Deng et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>Polygala tenuifolia</td>
<td>PC12 cells overexpressing α-syn (A53T)</td>
<td>12.5–50 μM</td>
<td>↑ Autophagy flux</td>
<td>AMPK/mTOR dependent</td>
<td>(Wu et al., 2013)</td>
</tr>
<tr>
<td>Trehalose</td>
<td>Various plants</td>
<td>PC12 cells overexpressing α-syn (WT, A53T and A30P)</td>
<td>100 mM</td>
<td>↑ Autophagy flux, ↓ α-syn</td>
<td>mTOR independent</td>
<td>(Sarkar et al., 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PC12 cells treated with rotenone (WT, A53T and A30P)</td>
<td>100 mM</td>
<td>↑ Autophagy flux, ↓ α-syn</td>
<td>TFEB activation</td>
<td>(Wu et al., 2015)</td>
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<tr>
<td></td>
<td></td>
<td>Lewis rats treated with rotenone (2 mg/kg, s.c. injection)</td>
<td>2%, w/v</td>
<td>↑ TH-positive neurons</td>
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<td></td>
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<td>AAV1/2 A53T α-syn-injected SD rats</td>
<td>2%–5%, w/v</td>
<td>↓ Behavioral impairment, ↑ TH-positive neurons, ↑ dopamine, ↓ α-syn aggregates, ↑ Autophagy flux</td>
<td></td>
<td>(He et al., 2016)</td>
</tr>
<tr>
<td>Onjisaponin B</td>
<td>Polygala tenuifolia</td>
<td>PC12 cells overexpressing α-syn (WT, A53T and A30P)</td>
<td>100 mM</td>
<td>↑ Autophagy flux, ↓ α-syn</td>
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</table>
Triterpene

Celastrol. Celastrol is a triterpenoid found in the extract of Tripterygium wilfordii. Celastrol has been reported to induce autophagy and apoptosis in tumor cells via the ROS/JNK pathway (Li et al., 2015). Meanwhile, whether celastrol has neuroprotective effects has been investigated. Celastrol could protect SH-SY5Y cells from rotenone-induced injuries by enhancing autophagy (Deng et al., 2013). Another triterpene, onjisaponin B, isolated from Radix Polygalae has been reported to enhance autophagy and to promote degradation of mutant a-synuclein in PC12 cells (Wu et al., 2013). Mechanistically, onjisaponin B induces autophagy via the AMPK-mTOR signaling pathway (Wu et al., 2013).

Flavonoids

Kaempferol. Kaempferol is a flavonoid found in fruits and vegetables such as Cuscuta chinensis and Hypericum perforatum. In cancer cells, kaempferol induces autophagy through AMPK and AKT signaling (Huang et al., 2012). Kaempferol has been shown to exert neuroprotective effect in a rotenone model of PD by enhancing autophagy-mediated mitochondrial turnover (Filomeni et al., 2012).

DISCUSSION AND CONCLUSION

Methodological quality/risk of bias

We have summarized the protective effects of major natural ALP enhancers in cellular and animal models of PD (Table 1). These natural compounds vary in structure and regulate different pathways in the ALP, either mTOR dependent or independent. However, some studies reporting the neuroprotective effects of natural compounds, such as paeoniflorin, quercetin and β-asarone on PD models, are not included in this review due to the defective methodology used and, therefore, risk of bias (Table 2). To determine whether a testing compound promotes autophagy flux, multiple assays should be conducted, including assessment of LC3 and SQSTM1/p62 turnover, according to widely recognized guidelines for monitoring autophagy (Klionsky et al., 2016). For the autophagy marker LC3, the increase in LC3-II may reflect enhanced autophagosome formation or impaired lysosomal degradation. It is very important to conduct immunoblot and fluorescent assays of LC3 in the presence of genetic or pharmacological inhibitors of ALP to determine the LC3 flux (Klionsky et al., 2016). For SQSTM1 turnover assays, the levels of SQSTM1 do not always correlate with autophagy flux. The mRNA and protein levels of SQSTM1, the detergent-soluble and insoluble forms SQSTM1 should be comprehensively determined to draw a valid and reliable conclusion (Klionsky et al., 2016). Furthermore, the function and activity of lysosomes should also be considered when screening ALP enhancers. The studies with defective methodology are listed in Table 2. Although the tested compounds showed neuroprotective effects in cellular and (or) animal models of PD, the observed effects cannot be directly linked to the autophagy-regulating activity of the compounds because the autophagy markers (LC3-II and p62) were not determined in the cells or animals treated with or without the compounds in most studies (Table 2). One study determined the levels of LC3-II and p62 in rats brains treated with paeoniflorin. However, no changes of LC3-II and p62 by paeoniflorin treatment in the sham-operated rats were indicated (Gu et al., 2016), which cannot support the conclusion that the neuroprotective effects of paeoniflorin are mediated by autophagy.

CONCLUSION

Current pre-clinical studies suggest that targeting the ALP by natural products may be an alternative and promising strategy for the prevention and treatment of PD. For future studies, the direct molecular targets of natural ALP enhancers need to be investigated, although the molecular pathways involved have been indicated. Last and most importantly, the in vivo efficacy of these ALP modulators should be comprehensively evaluated in multiple genetic and neurotoxic models of PD.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Animals/cells</th>
<th>Models</th>
<th>Dose, route and time/ concentration and time</th>
<th>Outcomes</th>
<th>Autophagy markers</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeoniflorin</td>
<td>SD rats</td>
<td>6-OHDA</td>
<td>60 mg/kg/day</td>
<td>Motor dysfunction, ↑α-syn, ↑TH</td>
<td>LC3-II p62</td>
<td>(Gu et al., 2016)</td>
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<tr>
<td></td>
<td></td>
<td>injection × 3 weeks (i.p.)</td>
<td></td>
<td></td>
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<tr>
<td>PC12 cells</td>
<td></td>
<td>MPP +</td>
<td>50 μM, 24 h</td>
<td>Cell death, ↑α-syn</td>
<td>N.D.</td>
<td>(Sun et al., 2011)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Albino rats</td>
<td>Rotenone</td>
<td>50 mg/kg/day</td>
<td>Behavioral dysfunction</td>
<td>N.D.</td>
<td>(El-Horany et al., 2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>injection × 3 weeks (i.p.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-asarone</td>
<td>SD rats</td>
<td>6-OHDA</td>
<td>10–40 mg/kg/day</td>
<td>Behavioral dysfunction, ↑α-syn, ↑TH</td>
<td>N.D.</td>
<td>(Zhang et al., 2016)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>injection × 4 weeks (p.o.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN4741 cells</td>
<td></td>
<td>6-OHDA</td>
<td>10–100 μM, 24 h</td>
<td></td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

Notes: ↔ indicates no changes; N.D. indicates ‘not determined’.
Acknowledgements

This study was supported by the grants of RGCHKBU-121009/14, HRMF1232091, RC-IRMS/15-16/04, FRG 1/15-16/042 and FRG 2/15-16/034 (to Min Li) and FRG 1/16-17/019, FRG 2/16-17/019 and RC Start up grant for new academics (38-40-183) of Hong Kong Baptist University (to Ju-Xian Song). The authors would also like to thank Dr. Martha Dahlen for her English editing of this manuscript.

Conflict of Interests

The authors declare that there is no conflict of interest.

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