What are the Prospects of Treating Neurodegenerative Diseases with Natural Products?

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Abstract

The aetiology of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, is not fully understood. This is due to the fact that the development of such diseases is complex and multi-factorial. To date, several studies highlighted that one of the main mechanism of neuronal cell death is oxidative stress. This led researchers to investigate several naturally-occurring compounds, including dietary metabolites, and relate their mode of action to the oxidative stress mechanisms postulated in neuronal cells. In this review, we discuss the potential role of natural metabolites as candidates for the control and treatment of neurodegenerative disease, with a particular reference to Alzheimer's disease and Parkinson's disease.

Keywords: Degenerative Diseases; Alzheimer's Disease; Parkinson's Disease; Neuronal Cells; Natural Products

Introduction

In recent years, research demonstrated evidence that neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) result, in part, from oxidative stress exerted on neuronal cells. The mechanism of cell death commences with a high concentration of reactive oxygen species (ROS) that leads to the dysfunction of mitochondria. In turn, this may result in the oxidation and aggregation of protein [1,2]. Under normal situations, the effects of moderate concentrations of ROS, leading to protein aggregation effects, are counteracted by the autophagy-lysosomal pathway [3,4]. Autophagy leads to the packing of defective cytoplasmic material in vesicles that are delivered to lysosomes for degradation. However, in AD and PD, excessive ROS affects also this repair system leading to the accumulation of dysfunctional mitochondria and more protein aggregates which will lead to neuronal cell death. To a lesser extent, another damaging reactive species is the reactive nitrogen species (RNS).

Oxidative stress, related to ROS, is a combination of several pathways and processes that provide a synergistic negative effect on the physiological functions of cells (Figure 1). This review aims to simplify the oxidative stress resulting from ROS, so as to reach a wide readership beyond neurological societies. In principle, the main source of ROS originates from the mitochondria. During respiration, the incomplete reduction of oxygen results in the formation of anionic radicals that may leak into the surrounding cytoplasm. It has been found that under normal conditions, a very small percentage of oxygen (1 - 3%) is transformed into superoxide in the mitochondria. As the mitochondrion is the site of energy production, over 85% of the oxygen, entering the cell, is used within this organelle, and consequently a high amount of ROS is produced. The remaining percentage of oxygen is used by cytoplasmic enzymatic processes, such as the xanthine oxidase system and other enzymatic systems at the cytochrome P450 of the endoplasmic reticulum [5]. Through these systems superoxide and hydrogen peroxide are generated, which though are both weak oxidising agents, they generate the peroxynitrite (via nitric oxide synthase) and hydroxyl radicals, respectively. The presence of transition metals, such as iron (II), accelerate the second type of

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reaction. Indeed, alterations in iron levels appear to be specific to the *substantia nigra pars compacta*, the mesencephalic area affected by dopamine depletion in PD [6]. The generation of RNS and ROS result in the damage of cellular structures such as nuclear and mitochondrial DNA damage and mutagenesis, protein damage and defective protein metabolites, and the oxidation of unsaturated fatty acids in the cell membrane. These mechanisms enhance mitochondrial DNA mutagenesis and ultimately cell death due to a defective containment of energy processes.
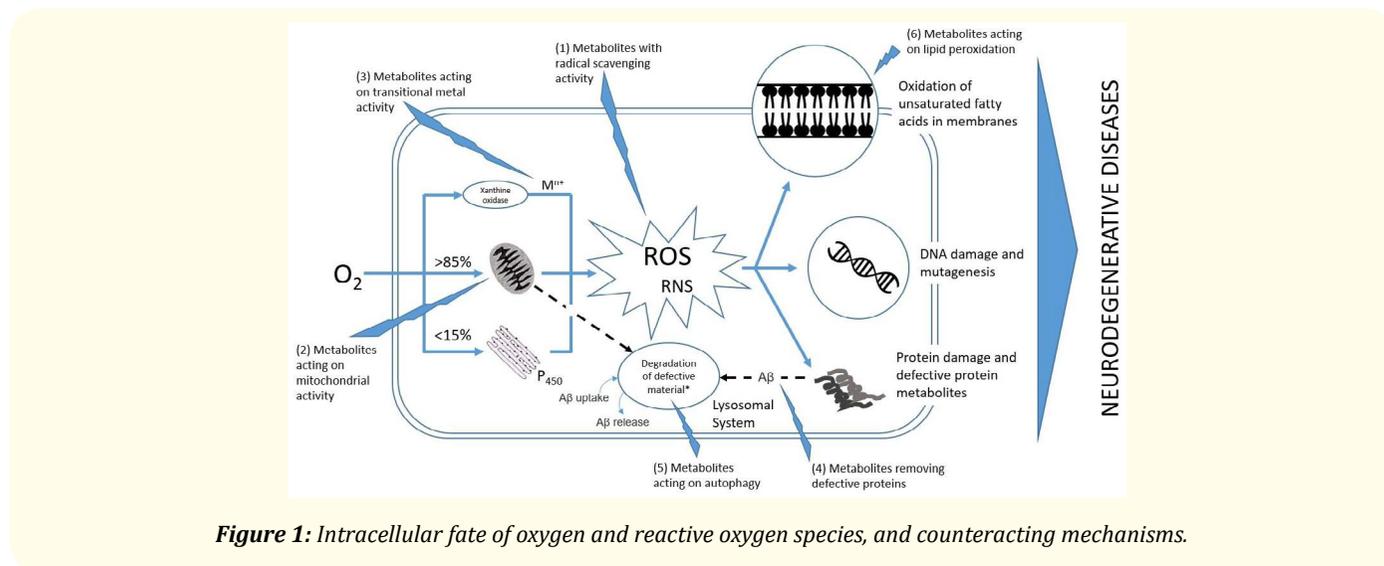

Figure 1: Intracellular fate of oxygen and reactive oxygen species, and counteracting mechanisms.

In response, the cellular mechanism counteracts the physiological concentrations of reactive species produced, thus limiting cellular damage and cell death. Mechanisms that counteract the damage include: (a) scavenging of reactive species by dietary and endogenous low molecular weight substances, outside and inside the cell; (b) the activity of antioxidant enzymes (peroxidase, catalase and superoxide dismutase) in the removal of reactive species; (c) decreasing the presence of transition metals; (d) the activity of heat-shock proteins in the removal of defective protein, or repair and reversibility of protein damage [5]; (e) the enhancement of the autophagous-lysosomal pathway and (f) the maintenance of cell membranes through the reduction of lipid peroxidation.

Therefore, the search for natural products that may interfere with the degenerative pathway, led to the discovery of a number of plant metabolites with such effects. However, the mechanism of action varies from one group to another. *In vitro* neuronal degenerative studies have been conducting using both short-term neuronal cultures [7] and immortalised cell lines [8-10]. Neurodegenerative studies have been also carried out in non-mammalian models such as the expression of the Aβ peptides, parkin and Lrrk mutant in *Drosophila melanogaster* [11-13]. Current therapy aims at relieving symptoms or slowing down the neurodegeneration. However, this form of therapy does not necessarily improve the quality of a patient suffering from AD or PD. Consequently, a number of natural products have been tested on AD and PD under *in vitro* conditions to determine their effects on the autophagy-lysosomal pathway with the aim of preventing or stopping neuronal cell death [14-17].

This review provides an insight in the natural products to date that have been tested on different AD and PD intracellular models, and discusses the role of plant-derived natural products in complementing the six counteracting mechanisms as illustrated in Figure 1.

Mechanisms counteracting neuronal damage

Dietary low molecular weight substances with scavenging activity against reactive species

Several studies on the antioxidant activities of several plant extracts and single constituents have been conducted, by several research groups, so far. These effects can take place either outside or inside cells. The scavenging of free radicals is a very general pharmacologi-

cal activity and this type of activity is relatively non-specific with implications on other biological systems. However, research conducted on neurodegenerative disease, did not solely describe the antioxidant activity of metabolites on neuronal cells but also encompassed other assays related to neurodengenerative diseases [18,19]. The most quoted assay to determine the strength of scavenging activity is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Curcumin and derivatives exhibited an antioxidant activity of less than 36.2 µg/ml [14], while isoflavonoids exhibited activities of less than 34.7 µg/ml [19]. Other flavonoids, anthocyanins from *Vitis vinifera*, exhibited values less than 850 µg/ml [20]. The chloroform extract from *Feronia limonia* bark, containing 7-hydroxycoumarin, exhibited a scavenging activity of 17.4 µg/ml [21]. An ethanolic extract from *Inula helenium* exhibited an activity less than 60 µg/ml [10], however the active metabolites were not characterised. Non-plant natural sources particularly honey and propolis exhibit scavenging activity against reactive species [22]. Honey and its flavonoids exhibited scavenging activities ranging 3.326 - 11.865 mg/100 g honey with flavonoid contents of 0.655 - 212.865 mg/100g honey [23]. In another study, the identified honey flavonoids with antioxidant activity include mainly chrysin, kaempferol and quercetin [24]. Russo and coworkers [25] showed that caffeic acid phenethyl ester and galangin in propolis exhibited scavenging activity. Several other studies report the antioxidant activity of a number of natural sources [26,27].

Metabolites that enhance the activity of intracellular antioxidant enzymes

Plant metabolites typified by this property enhance the activity of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase [28]. Such metabolites reduce the high quantity of reactive species, hence reducing the impacts of ROS and RNS. The production of mitochondrial ROS is impaired via autophagy by cucurbitacins [17,29,30]. Oleuropein, a seciridoid glycoside found as a major phenolic in the olive [31,32], enhanced such enzymes in *in vivo* models as well as 24-epibrassonolide in a cellular model of PD [33]. This activity is also exhibited by other phenolics, such as quercetin and kaempferol in extracts of *Moringa olifera* [34]. A standardized extract of *Scrophularia buergeriana* exhibited a similar activity [35]. Another standardised extract, from *Bacopa monniera* increased superoxide dismutase, catalase and glutathione peroxidase activities in frontal cortex, striatum and hippocampus [36]. Vitamins play an important role in assisting intracellular antioxidant enzymes against oxidative stress induced by radicals. In particular, vitamins C and E, as co-factors with selenium, assist glutathione peroxidase against hydroxyl radicals and lipid peroxide. Moreover, vitamin C as co-factor with copper, zinc and manganese assists superoxide dismutase against superoxide radicals and as co-factor with iron supports catalase against hydrogen peroxide [26]. Fruits and vegetables are good sources of vitamins [37].

Metabolites that decrease the presence of transition metals

The antioxidant activity of metabolites has been also investigated using assays directed at reducing and chelating transition metals and other heavy metals under *in vitro* conditions. A typical assay is the ferric reducing ability of plasma (FRAP) [38]. The essential oil from lemon peels exhibited ferric reducing and chelating effects. Typical metabolites include sabinene, limonene, pinene amongst others [39]. In a study, the antioxidant reducing power of honey was less than 16.6 mg honey/ml [23]. Dudonne and coworkers [27] found a number of plant extracts with significant metal scavenging activity, including oak, clove, cinnamon, and pine extracts (6.45 - 15.92 mmol Fe^{2+}/g). In another study, the essential oil of *Cistus libanotis* showed a value of 19.2 µM Fe^{2+}/g [40].

Metabolites that assist in the removal of defective proteins

Amongst the natural products that assist in the removal of defective proteins, several researchers mention dietary heat-shock proteins. However, research also highlights the potential of certain natural products to induce heat shock response [41]. Curcumin is one of the constituents of turmeric that is used as a food supplement for neurodegenerative diseases. It also binds to small Aβ oligomers preventing aggregation with other molecules [42]. Celastrol, a quinone methide triterpene, exhibits heat shock activation by inhibiting glucocorticoid receptor activity [43]. Epigallocatechin-3 gallate (EGCG), the major polyphenol found in green tea, exhibited fibril destabilizing effects *in vivo* in AD and PD models [44]. In another study by Yan and coworkers [45], paeoniflorin was identified as a heat shock protein–inducer while glycyrrhizin as a co-inducer, in the presence of heat shock. Several other studies show the effects of heat shock protein-induction in various other contexts such as ischaemia, inflammation and infection [46,47].

Metabolites that maintain the lysosomal system.

The endosomal-lysosomal system is also involved in the uptake and production of amyloid-beta (Aβ), which plays an important role in neurodegenerative pathogenesis [48]. This system removes defective mitochondria. There is a number of natural products which interact with this system by decreasing Aβ levels. A particular group of terpenoids, cucurbitacins [49], assist the lysosomal system by maintaining its distribution and decrease excessive autophagic activity [50] in a cellular model of PD. Excessive autophagy should not be allowed inside a cell as this may be detrimental to the cell itself. Several other natural metabolites interfere with mechanisms related to the intracellular Aβ levels. Flavonoids, such as quercetin, apigenin and baicalein, inhibit the formation and aggregation of Aβ [17,51,52]. Another activity is the clearance of Aβ by resveratrol [53], hence reducing the intracellular Aβ burden.

Metabolites that reduce lipid peroxidation

Several researchers report that lipid peroxidation results from the presence of Aβ as a consequence of high intracellular free radical concentrations [54]. Although treatment aims at targeting the cause of damage, i.e. free radical formation, secondary therapeutic agents are developed in order to assist in counteracting the damage provoked by the cause. Lipid peroxidation is usually measured *in vitro* by the thiobarbituric acid reactive substances (TBARS) assay. Chonpathompikunlert and coworkers [55] postulated that piperine, an alkaloid obtained from pepper species reduced the damaging effects of lipid peroxidation. In another study, the chloroform extract from *Feronia limonia* bark, containing 7-hydroxycoumarin, exhibited a lipid peroxidation scavenging activity of 14 µg/ml [21] which was more potent than the standard catechin. In addition, ECGC and quercetin showed to reduce lipid peroxidation in *in vitro* and *in vivo* model of PD [56]. A powerful natural product that inhibits lipid peroxidation is tocopherol [57]. When phospholipid bilayers were challenged with ROS, *in vitro*, the flavonoids (−)-epicatechin, (−)-epicatechin gallate and quercetin counteracted lipid peroxidation [58,59]. However, it is believed that such activities are possible by the synergistic action of natural metabolites. For example, the effects of a extract of *Ginkgo biloba* on cognitive disorders results from the synergistic activity of the flavonoids, the terpenoids (ginkgolides, bilobalide), and the organic acids found in the extract [60].

Conclusions

Though not exhaustive, this mini-review shows the potential use of natural products in neurodegenerative diseases. Nature offers a plethora of natural metabolites that may be potentially effective in the prevention and control of neurodegenerative diseases. Although to date, research with natural products is presented with the effective testing of single metabolites, more work has to be done to primarily characterise the crude extracts and test more metabolites in such biological systems. Such studies may bridge the traditional uses of crude extracts in the control of neurological conditions with evidence-based rationalisation of the efficacy of active metabolites.

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