10. Neuroprotective effects of oxyresveratrol from fruit against neurodegeneration in Alzheimer’s disease

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Abstract. Many countries are stepping into aging society. The increasing proportion of elderly in population is in high risk of having more aging-associated diseases. Alzheimer’s disease (AD) is the most common age-associated disorder, characterized by progressive dementia. Nowadays, 30 million people have suffered the disorder. However, no cure is applied in clinic except symptom-relieving strategy. Epidemiological studies have shown the reverse association between consumption of nutritional diets rich in anti-oxidants and developing neurodegenerative diseases. Therefore, healthy aging should be put forward by introducing healthy life style, in which good nutrition plays a key role.

Oxyresveratrol (OXY) is a natural hydroxystilbene present in mulberry fruit (fig.1), or fruits of several trees belonging to \textit{Artocarpus} genus. It has been found as tyrosinase inhibitor.
Recently, increasing lines of evidence have demonstrated its neuroprotective effects on age-associated diseases such as AD and stroke. Apart from conventional antioxidant property and the permeability to blood-brain-barrier, we focus on reviewing the anti-inflammatory activity, cell signaling regulation property, as well as its bioavailability in vivo and in vitro. Findings enlighten that nutritional supplement, such as fruits containing OXY may retard aging or benefit healthy aging, which is helpful to reduce the risk of neurodegeneration in elderly population.

Introduction

Dementia and Alzheimer’s disease (AD)

In the next few decades, with the increasing longevity in population, most Western and Asian countries are stepping into elderly society. Meanwhile, a ‘silent epidemic’ is spreading with various problems from elderly community [1]. Dementia has been one of the major public health problems in both developed and developing countries. The prevalence of senile dementia increases dramatically from 0.8% in the 65–69-year-old groups, and to 28.5% in populations of 90 years and older [2]. In developed countries, the prevalence rate of dementia among people aged ≥ 65 years has been reported to be around 5–6%. Among all types of dementia, AD is the commonest form, with a prevalence of 0.4% in women and 0.3% in men aged 60–69 years, rising to 11.2% in women and 10% in men over 80 years [3]. Systematical investigation has revealed that the chronological prevalence of AD increased significantly from 1980 to 2004 in China. As the most major subtype of dementia in China, the pooled prevalence of AD for the population aged 60 years and older is 1.6% [4].

In the process of aging, the brain appears more and more vulnerable to oxidative stress, which has been reported to be the major culprit of the most commonly age-related neurodegenerative disease, AD. The onset and manifestation of AD would significantly decrease the quality of life in human, along with psychiatric and behavioral problems (e.g. depression, delusions, misidentifications), and cognitive deficiency that results in aphasia (impairment of language), amnesia (loss of memory), agnosia (inability to recognize) and apraxia (incapability in motor behaviors). These deficiencies would gradually destroy daily life, such as difficulties in using telephone, dressing, driving, eating and toileting. Mental emptiness and loss of controlling whole body functions will eventually occur in the last stage of the disorder [5].

Risk factors leading to the development of AD include genes like the APP gene, the presenilin genes 1 and 2, the α2-macroglobulin gene and the apolipoprotein E-4 gene [6-8], as well as a series of factors concerned with
daily life, such as smoking, alcoholism, diet, high LDL cholesterol, depression, hypertension, even low level of education [9].

**Generation of reactive oxygen species (ROS), oxidative stress and AD**

Physiologically, ROS are normal intrinsic metabolites as utilizing O$_2$ by most life organisms. The narrow definition of ROS refers to oxygen free radicals and nonradical reaction products, including superoxide radical anion (O$_2^-$), hydroxyl radical (HO’), hydroperoxyl radical (HO$_2^-$) and hydrogen peroxide (H$_2$O$_2$). As normal metabolites, ROS have been documented for mediating intracellular signaling and redox regulation to keep cell homeostasis [10; 11]. ROS are reported to play roles in normal biochemical processes involving some growth factors, cytokines, hormones, as well as neurotransmitters [12-14]. On the other hand, organisms have developed defense systems to scavenge excessive ROS or quench them by transforming ROS into less active or safe products. The intrinsic antioxidant defense systems are operated in enzymatic and nonenzymatic ways, and the cellular antioxidants consist of (1) enzymes that can interact with ROS; (2) enzymes that can catalyze the generation or regeneration of cellular antioxidants [15]; (3) metal chelators, inhibiting reactions (Fenton reaction) catalyzed by metal and therefore preventing the generation of free radicals; and (4) low-molecular weight antioxidants such as glutathione (GSH), NADH, carnosine, uric acid, melatonin, belirubin. Besides endogenous anti-oxidants, several kinds of low-molecular weight antioxidants (e.g. phenolics, ascorbic acid, carotenoids, quinones, tocopherols) can be intaken by dietary supplement [16]. In this context, keeping stable redox status within life body is essential to be healthy, even in the process of healthy aging. Once the cellular balance is destroyed by severe burdens of ROS, the inability of intrinsic antioxidant defense systems, or both of the situations, oxidative stress occurs subsequently.

Under oxidative stress, the excessive production of ROS may directly damage proteins, lipids, carbohydrates, DNA and even cellular molecules involved in antioxidant defense systems. In addition, normal cellular signaling events may be interfered and dysregulated by excessive ROS. Increasing lines of experimental or clinical studies have proposed oxidative stress as the major pathological cause of a series of human diseases, such as cancer, cardiovascular diseases, diabetes, as well as age-associated immune and neurodegenerative disorders (AD, Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS)) [17; 18]. Among all cases, the central nervous system (CNS) seems to be particularly vulnerable to ROS. High consumption of total body oxygen (about 20%) by
the brain, high content of unsaturated lipids, iron accumulation in brain-specific regions but relatively deficiency in iron-binding proteins, as well as the limited ability of regeneration for brain tissues, may account for the vulnerability of brain to pro-oxidant environment [5].

**Antioxidants and AD**

Although the exact etiology of AD remains unknown, increasing lines of evidence have demonstrated the crucial role of oxidative stress in the pathogenesis of AD. Reports of oxidative damage in AD include lipid peroxidation, decreased unsaturated fatty acids, increased iron, aluminum, and higher levels of the most frequent oxidative modification of nucleic acid, 8-hydroxyguanosine (8-OHG) in AD brains [19; 20]. Increased ROS are proposed to be produced by activated microglia that surround most senile plaques, Aβ deposition in conjunction with binding of redox-active transition metals, protein modification by advanced glycation and lipoxidation end products (AGEs and ALEs), mitochondrial abnormalities such as reduction in mitochondrial electron transport and mutation in cytochrome c oxidase genes [21; 22].

A number of studies have compared the antioxidant concentrations between AD patients and controls, and find that the concentrations of antioxidants like Vitamin A, C, or E are decreased to different extents in the plasma, cerebrospinal fluid and brains of AD patients. A series of epidemiological studies have suggested the inverse association between dietary antioxidants intake and the development of AD or cognitive impairment. The dietary antioxidants mainly include Vitamin A, C, and E, red wine, tea and curry [16; 23-25]. The limitation for these reports is that only few of them are study from randomized double-blind clinical trials. Debate about the relationship between antioxidants intake and AD onset and disease progression become difficult to be resolved. This shifts the focus of a group of scientists to investigate healthy lifestyle to promote healthy aging with interventions in diet and exercise [26; 27]. A number of laboratories have demonstrated significant neuroprotective effects of berry (blueberry [28; 29], strawberry [30], cranberry [31], mulberry [32]) fruit polyphenols, dietary flavonoids [33], phenolic apple extracts [34], spinach extracts [30], and / or grape supplements [35]. Among various kinds of polyphenols, we here mainly focus on introducing biological and pharmacological activities, especially its neuroprotective effects, of oxyresveratrol (OXY, a natural stilbene analogue to resveratrol (RES)).

Stilbene is a class of antioxidant compounds sharing the same chemical skeleton, which is a diarylethene, a hydrocarbon consisting of an **trans/cis**
ethene double bond substituted with a phenyl group on both carbon atoms of the double bond. The name stilbene was derived from the Greek word stilbos, which means shining. Many stilbene and their derivatives (stilbenoids) are present naturally in plants (dietary fruits or herbs). The most widely investigated stilbene compound is resveratrol (3,5,4’-trans-trihydroxystilbene, RES). It is found to be a stilbene phytoalexin in plants such as grapes, peanuts, berries and pines [36]. RES is synthesized in these plants to counteract various environmental injuries such as UV irradiation and fungal infection. RES is reported to be one of the active agents in Itadori tea, which has been used as a traditional medicine mainly for curing heart disease and stroke in China and Japan [37]. Since epidemiological studies have reported the inverse association between moderate consumption of red wine and the incidence of coronary heart disease, RES as the major ingredient in red wine has stimulated investigations on its cardioprotective activity which is due to its free radical scavenging property [38]. Among the wide range of biological and pharmacological activities, RES has been received increasing attention for its chemopreventive effects. It is reported to inhibit the growth of several tumor cell lines, such as leukemic, prostate, colonic, breast and esophageal

Figure 1. Picture of mulberry fruit. The color of the fruit changes from white to dark red along with maturity difference.
cells generally through inhibiting tumor initiation, promotion and progression. Besides, RES has been demonstrated to inhibit platelet aggregation and lipid peroxidation, attributing to inhibit the activity of cyclooxygenase and hydroperoxidase.

In recent years, studies on the activity of RES have extended to animal models of CNS disorders or injury, such as AD [39; 40], PD, HD, cerebral ischemia, as well as traumatic brain injury (TBI). Increasing numbers of report have shown that acute chronic treatment of RES exhibits neuroprotective effects against colchicine [41] 3-nitropropionic acid [42] or trauma [43; 44] -induced cognitive and motor impairment, as well as hippocampal neuron loss. The underlying mechanisms mainly attribute to the antioxidant activity of RES to reduce the related oxidative stress, including reducing the elevated malondialdehyde (MDA), lipid peroxidation, nitrite, nitric oxide (NO) and xanthine oxidase (XO) levels, increasing the depleted GSH level and succinate dehydrogenase activity in the brains of rats [39]. In addition, RES administration elicits neuroprotective effects on cerebral ischemia-induced [45] neuron damage, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced [46] motor coordination impairment, hydroxyl radical overloading, and neuronal loss through free radical scavenging activity.

**Biological and pharmacokinetics studies on oxyresveratrol (OXY)**

However, recent studies have found that RES is not the most effective agent in regard with some biological and pharmacological activities. Investigations on differential bioactivities of OXY (2,4,3’,5’-trans-trihydroxystilbene) have provided increasing lines of evidence on this point. From the chemical name of OXY, we can figure that OXY has an extra hydroxyl group compared to RES, making it be a readily hydrogen-donor, which accounts for its notable anti-oxidant activity. OXY can be found in heartwood or fruits of *Artocarpus heterophyllus, Artocarpus lakoocha, Artocarpus gomezianus, Artocarpus dadah*, wood extracts or fruits of mulberry trees (*Morus australis, Morus alba L.*), fruits of *Melaleuca leucadendron*, rhizome of *Smilacis chinae*, as well as Egypt herb *Schoenocaulon officinale*. Water-soluble OXY is known for its antiviral [47], hepato-protective [48] activities [49]. OXY reduces production of Aβ by inhibiting β-secretase (BACE1) [50]. We then mainly review its activities in tyrosinase inhibition, anti-inflammation, neuroprotection and pharmacokinetics.
Tyrosinase inhibitor

The first bioactivity of OXY that has been found is that OXY is a potent tyrosinase inhibitor [51; 52]. Tyrosinase is a widely distributed enzyme in nature, catalyzing the rate-limiting step for biosynthetic pathway of melanin pigments. It exists in many organisms with slightly different forms. In animals, the deposition and distribution of melanin pigments determine the skin color. Abnormal accumulation of melanin pigments is responsible for hyperpigmentations including melasma, freckles, and senile lentigines [53; 54], while depigmenting agent such as kojic acid, can afford satisfactory improvement to subjects. Kim et al. [55] have demonstrated that OXY exhibited potent tyrosinase inhibition activity in order of OXY >> kojic acid ≒ RES > rhapontigenin ≒ 3,5-dihydroxy-4’-methoxystilbene regarding the IC$_{50}$ values. OXY showed a 45-fold stronger inhibitory effect on mushroom tyrosinase activity than RES. They further demonstrated that OXY works through noncompetitive inhibition of tyrosinase activity rather than suppression of both mRNA expression and protein synthesis of tyrosinase. Kojic acid is able to chelate copper at the active site of tyrosinase, which is regarded as its underlying mechanism of inhibiting tyrosinase activity. The analogue of OXY, RES has shown antioxidant activity by chelating copper in several biological systems [56]. Consequently, authors proposed that the potent inhibition on tyrosinase activity of OXY may be attributed to the similar ability of chelating copper.

Anti-inflammatory activity

Reports have documented the in-vitro and in-vivo anti-inflammatory effects of OXY isolated from Artocarpus heterophyllus [57], Artocarpus dadah [49], or mulberry wood. Mori Cortex is the dried root bark of Morus alba L., and has been widely used as an antitussive, antiphlogistic, anti-inflammatory and diuretic herb in China, Japan and Korea. Chung et al. [58] have isolated mulberroside A and OXY from Mori Cortex extracts, the two compounds exhibit an in vivo anti-inflammatory effect on carrageenin-induced paw edema in rats, suggesting these compounds as the active components of Mori Cortex. They further investigated the underlying mechanism of the action by examining the effect of OXY on NO production and prostaglandin E$_2$ (PGE$_2$) biosynthesis in lipopolysacchride (LPS)-activated RAW 264.7 macrophage. The production of NO and PGE$_2$ have been implicated in the process of inflammation. Results exhibited that OXY could significantly inhibit the production of nitrite, PGE$_2$, the expression of inducible nitric oxide synthase (iNOS) and NF-κB activation. In contrast to
the *in vitro* study, Mouihate *et al.* [59] found that OXY blocked specifically LPS-induced hypothermia but had no significant effect on fever in rats. They show for the first time that OXY targets specifically TNF-α independent of IL-6 production or IL-6-activated STAT-3 signaling pathway [59]. However in the *in vivo* model, OXY did not alter the immune activation of NF-κB transcription factor in the liver; it did not affect the cyclooxygenase (Cox-2, a key enzyme in the production of inflammatory prostaglandin), and induction in the organum vasculosum of the lateralis terminalis (OVLT) / preoptic area (POA). The conflicting results may be attributed to the fact that inflammatory responses *in vivo* are much more complex than that *in vitro*. The cytokine specific property of OXY may suggest that it can target TNF-α production at phases of transcription, translation or secretion. All these anti-inflammatory effects may have implication as neuroprotective agent in AD.

**Neuroprotective effects**

OXY has been reported to exert neuroprotection in Aβ (25-35)-induced neurotoxicity in cortical culture neurons, 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in SH-SY5Y cells, as well as *in vitro* and *in vivo* models of transient cerebral ischemia.

In cultures, Aβ or Aβ peptide fragments can induce cell death and render neurons vulnerable to excitotoxicity and oxidative damage. N-methyl-D-aspartate (NMDA) receptor (a glutamate receptor subtype) modulation induced by glutamate release, sustained elevations of intracellular Ca$^{2+}$ concentration, and oxidative stress are proposed to be involve in mechanisms of Aβ-induced toxicity. It has been reported that OXY (effective dosage range 1-10 µM) isolated from *Smilacis chinae* rhizome can significantly inhibit 10 µM Aβ (25-35)-induced neuronal cell death by attenuating the elevation of cytosolic Ca$^{2+}$ concentration, glutamate release into medium and ROS generation [60].

6-OHDA is a neurotoxin widely used in PD culture model to induce oxidative stress to neurons [61-64]. Its capability of being transported into dopaminergic neurons via dopamine transporter and subsequent induction of free radical-mediated oxidation was proposed to be responsible for its neurotoxicity. Our group [32] revealed that dietary OXY (1-50 µM) elicited potent neuroprotective effects on 6-OHDA-triggered neurotoxicity by attenuating release of LDH and caspase-3-like activity, with a wider effective window compared to RES (1-10 µM). We further elucidated that OXY was able to pass through cell membrane (by HPLC analysis) and acted as an intracellular ROS scavenger. Furthermore, we demonstrated that the inhibition of 6-OHDA-activated JNK pathway and increase in cytosolic
levels of SIRT1 may account for neuroprotective effects of OXY. Our results have demonstrated the potential of OXY as an ideal neuroprotectant against neurodegeneration in PD.

Neurotoxicity initiated by overstimulation of NMDA receptors and subsequent influx of free Ca$^{2+}$ leads to an intracellular cascade of cytotoxic events. Ca$^{2+}$-dependent depolarization of mitochondria may contribute to oxidative stress in neuronal injury through production of ROS. NMDA neurotoxicity models have also been widely used to elucidate cellular responses to brain ischemia in vitro. Ban et al. [65] have demonstrated that *Smilacis chinea* rhizome (30 and 50 mg/kg, orally) prevented cerebral ischemic injury in rats induced by 3-h middle cerebral artery occlusion (MCAO) and 24-h reperfusion. They further showed that over a concentration range of 10-50 µg/ml *Smilacis chinea* rhizome protected neurons against 1 mM NMDA-induced cell death, increases in Ca$^{2+}$ concentration and ROS generation. They proposed that OXY and RES may be responsible for the effects of *Smilacis chinea* rhizome, since among six compounds isolated from *Smilacis chinea* rhizome, OXY and RES exerted neuroprotective effects similar to that of *Smilacis chinea* rhizome. In addition, a recent investigation also showed that an intraperitoneal application of a dose of 10 mg/kg OXY was sufficient to significantly provide neuroprotection against MCAO-induced brain infarct volume. The maximal protective capacity was at a dose of 20 mg/kg, since no further neuroprotection was seen by increasing the dose to 30 mg/kg. The cytochrome c release, immunohistochemical staining for caspase-3 as well as labeling of apoptotic-DNA were also found to be reduced after OXY treatment [66]. The authors proposed that the neuroprotective effects by OXY may be attributed to a combined effects of both its anti-oxidative and anti-nitrosative activities [67].

The anti-oxidant and neuroprotective activities of OXY attract an interesting research direction about whether OXY can reduce the toxicity triggered by elevated homocysteine (Hcy). Normal level of Hcy is involved in numerous methyl group transfer mechanisms, such as reactions targeting DNA, RNA, proteins, phospholipids and neurotransmitters. While elevated Hcy (> 14 µmol/L) in plasma, namely hyperhomocysteinemia (HHcy), has been reported to be an independent risk factor in systemic vascular diseases [68], cognitive impairment [69], dementia (including AD) [70-74] and PD [75; 76]. The major mechanisms underlying toxicity of HHcy may involve oxidative stress [77-80] because of the pro-oxidant property of excess amino acid, leading to endothelial damage or apoptosis proved in several cell lines [81-83]. Experimental studies have demonstrated that Hcy and its derivatives (homocysteic acid and homocysteic sulfinic acid) may induce excitotoxicity by stimulating NMDA receptors and damaging neuronal DNA, in cultured neurons of hippocampus [84]. In addition, *in vitro* studies show that HHcy induced by a diet lacking folate, or direct
infusion of Hcy may not exert direct neurotoxicity in mice, but might enhance the toxicity induced by neurotoxins, such as kainite [85] or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [86].

In recent years, some studies have reported elevated plasma Hcy levels in PD patients treated with L-3,4-dihydroxyphenylalanine (L-Dopa) [87], or even early PD patients [88]. Besides, HHcy in PD is reported to be related to B-vitamin status [89] and genetic factors [90; 91]. Although the question whether L-Dopa-treated PD patients with HHcy are at higher risk to develop vascular diseases and cognitive impairment remains to be solved, counteracting elevated Hcy in PD patients by effective agent (e.g. catechol-O-methyltransferase inhibitors, currently used in PD treatment) or dietary intake (folate and B-vitamins) should be part of therapeutic strategy, at least as improvement in general metabolic balance. Since OXY has exhibited significant anti-oxidant capacity and neuroprotective potential, observations above motivate us to explore whether OXY or related dietary supplement can reduce neurotoxicity induced by HHcy in vitro, in animal models, or in L-Dopa-treated PD patients.

Pharmacokinetics study

Early pharmacokinetics study [92] reported that, when Mori Cortex extracts were administered orally to rats, the bioavailability of mulberroside A was only about 1%. Most mulberroside A was converted into OXY and transported into the circulating blood, and the absorption ratio of OXY was estimated at about 50%. Recently, a sensitive and simple HPLC method [93] has been developed and validated for the determination of OXY and RES in rat plasma. After orally administered with 1 g/kg *S. china* extract (an equivalent to 180 mg/kg OXY and 80 mg/kg RES). Results showed that the two stilbenes were rapidly absorbed into the body fluid from the gastrointestinal tract (the $t_{max}$ was 15 min) and they could still be detected in the plasma at least 360 min after oral treatment. In vivo microdialysis [94] in the striatum showed that OXY could penetrate, to a low extent, the blood-brain barrier (BBB) in control animals, while microdialysis samples from animals that were subjected to MCAO displayed strongly increased OXY levels (more than six-fold) in the infarct region as compared to sham-operated rats, suggesting that OXY exerted neuroprotection by directly penetrating into the BBB.

Conclusions

Taken together, water-soluble OXY is widely present in fruits of *Artocarpus* plants, mulberry, and several herbs. OXY is reported to be more
effective in anti-oxidation, anti-inflammation and neuroprotection than widely investigated RES. Dietary OXY or fruits containing OXY may be good choices for constructing healthy lifestyle or dietary strategies promoting healthy aging. Moreover, OXY exhibits high bioavailability after oral administration to rats, and it can penetrate BBB to exert neuroprotection directly in transient ischemia model. However, no in vivo study has investigated its neuroprotective effects in AD or PD. To develop much more effective neuroprotectant in AD or PD, further animal work deserves exploring for OXY.

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References