Neuroprotective action of resveratrol

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A B S T R A C T

Low-to-moderate red wine consumption appeared to reduce age-related neurological disorders including macular degeneration, stroke, and cognitive deficits with or without dementia. Resveratrol has been considered as one of the key ingredients responsible for the preventive action of red wine since the stilbene displays a neuroprotective action in various models of toxicity. Besides its well documented free radical scavenging and anti-inflammatory properties, resveratrol has been shown to increase the clearance of beta-amyloid, a key feature of Alzheimer’s disease, and to modulate intracellular effectors associated with oxidative stress (e.g. heme oxygenase), neuronal energy homeostasis (e.g. AMP kinase), program cell death (i.e. AIF) and longevity (i.e. sirtuins). This article summarizes the most recent findings on mechanisms of action involved in the protective effects of this multi target polyphenol, and discusses its possible roles in the prevention of various age-related neurological disorders. This article is part of a Special Issue entitled: Resveratrol: Challenges in translating pre-clinical findings to improved patient outcomes.

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1. Introduction

It is well known that a healthy diet may delay the occurrence of age-related neurological disorders such as Alzheimer’s and Parkinson’s diseases and stroke [1,2]. Hence, it has been reported that regular consumption of fruits, vegetables and fish lowered the risk of cognitive decline related or not to dementia in the elderly population [1]. Moreover, older adults who consume red wine (up to three glasses per day) on a regular basis reduced (up to 50%) the risk of developing dementia, such as Alzheimer’s disease (AD) and vascular dementia, as well as macular degeneration [1,3,4]. This is of particular interest since the number of people that will be at-risk or affected by age-related neurodegenerative disorders will rise, resulting in an increase in economic burden of care and treatment of patients.

Scientists have intensively investigated the mechanisms of action by which protective diet, and particularly red wine, prevents age-related neurodegenerative diseases. Although there are numerous polyphenols present in red wine that may be responsible for its beneficial effect, resveratrol has drawn particular attention because it is considered as a red wine-derived polyphenol with cardioprotective effects [5,6]. Numerous studies ranging from cell cultures to animal studies have demonstrated that resveratrol exhibits anti-inflammatory and antioxidant properties, inhibits beta-amyloid (Aβ) protein aggregation and modulates intracellular effectors involved in neuronal cell survival/death. More recently, resveratrol has been proposed to exert neuroprotective effects through activation of SIRT1, an enzyme that deacetylates histone H3 [7]. Here, we review possible mechanisms underlying the purported neuroprotective action of resveratrol in vitro as well as rodent models of diseases. See Table 1 and Fig. 1 for details.

2. Reactive oxygen species

Higher levels of reactive oxygen species (ROS) in aging were shown to be associated with cognitive deficits related or not to AD...
Resveratrol displays potent antioxidant activity because of its ability to scavenge free radicals and metals (i.e. copper) and to up-regulate endogenous antioxidant enzymes including glutathione peroxidase [9,10]. We have shown that resveratrol is able to protect and even rescue hippocampal neurons that were exposed to nitric oxide (NO), an effect that could be explained, at least in part, by its purported antioxidant activities, as well as its ROS scavenging properties [11].

Resveratrol may also play a beneficial role in the cellular response by modulating enzymes involved in stress response, such as quinone reductase 2 (Q2R), a cytosolic enzyme which enhances the production of damaging activated quinone and ROS [12]. Our group demonstrated that Q2R is overexpressed in the hippocampus – a brain area involved in learning and memory and severely affected in AD – in rat models of learning deficits, suggesting that the overexpression of this enzyme triggers memory impairments [13]. This hypothesis is supported by the fact that selective inhibitors of Q2R were able to block hippocampal neuronal cell death induced by menadione, a Q2R substrate [13]. Interestingly, resveratrol inhibits Q2R at low micromolar concentration (IC50 of 2.9 μM) [14], an effect that may lead to an increase in cellular resistance against oxidative stress-induced neuronal death [15]. Besides its inhibitory action on Q2R, resveratrol has been shown to induce heme oxygenase 1 (HO1), an endogenous enzyme that provides resistance against oxidative stress–related neuronal damage [16]. The authors first showed that resveratrol (25 μM) increased HO1 protein levels in primary neuronal cells exposed to glutamate, an effect that was due to an increase in HO1 protein synthesis. Moreover, a pre-treatment with resveratrol (20 mg/kg) significantly attenuated infarct size in wild-type but not HO1 knockout mice exposed to ischemia, suggesting that the polyphenol also interacts in vivo with the enzyme [16]. Using neuronal cell lines, Dasgupta and Milbrandt [17] showed that resveratrol is a potent activator of AMP-activated protein kinase (AMPK), a key regulator of cell survival in response to oxidative stress insults [18]. Moreover, resveratrol was shown to protect mitochondria against oxidative stress induced by a mixture of arachidonic acid and iron, through a mechanism that involves AMPK-dependent phosphorylation of glycogen synthase kinase-3beta (GSK3β) [19]. These authors also reported that the neuroprotective action of resveratrol depends on the presence of liver kinase B1 (LKB1), an upstream kinase of AMPK [19]. Furthermore, this LKB1-dependent mitochondrial protection resulted from resveratrol’s poly(ADP-ribose)polymerase activation, but not SIRT1 activation [19].

### 3. Neuroinflammation

Microglial activation may contribute to neuronal death during brain damage by releasing neurotoxic pro-inflammatory molecules [20]. It has been reported that resveratrol inhibits the pro-inflammatory molecules known as cyclooxygenases, particularly cyclooxygenase-1 (COX1) (for review see [10]), an enzyme involved in the production of pro-inflammatory molecules known as cytokines. Moreover, Jin et al. [21] showed that resveratrol exerted an in vivo neuroprotective action against toxicity induced by 6-hydroxydopamine (6-OHDA), and this effect was associated with its ability to reduce expression of COX-2 and tumor necrosis factor α (TNFα) in the substantia nigra. Resveratrol is also able to reduce the release of pro-inflammatory factors through the inhibition of cellular cascade signaling pathways involving nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) [22]. Using rat primary microglia cultures exposed to lipopolysaccharide (LPS), it has been reported that resveratrol (up to 50 μM) reduced the production of prostaglandins (e.g. PGE2), NO, and TNFα, as well as the expression of COX-1 and activation of NF-κB [23–26]. Finally, it has been shown that resveratrol (15–60 μM) protected against dopaminergic neuronal death induced by LPS by attenuating the activation of mitogen-activated protein kinases (MAPKs) and NF-κB signaling pathways, supporting the hypothesis that neuroprotection triggered by resveratrol involved inhibition of microglia-mediated neuroinflammation [27].

Astrocytes, a type of glial cells, actively regulate brain energy metabolism, neurotransmitter release, ion homeostasis and defense against oxidative stress [28,29]. Using a model of astrocytes exposed to H2O2, Quincozes-Santos et al. [30] have reported that resveratrol (100 μM) decreased nitrate and nitrogen species levels, whose overproduction likely plays a role in Alzheimer’s and Parkinson’s diseases [31]. Moreover, resveratrol enhanced per se HO1 expression and reduced ROS production possibly through its HO1 in these cells [30]. HO1 can also reduce NF-κB activation (for review, see [32]). Finally, it has been recently shown by the same group that resveratrol stimulated antioxidant defenses by increasing glutathione levels and decreased cytokines in rat hippocampal cultured astrocytes [33], a process that may explain its protective action against toxicity associated with neuroinflammation and be relevant to neurodegenerative diseases [34].

### 4. Amyloidogenesis

There is accumulative evidence suggesting that progressive accumulation of Aβ plaques plays a crucial role in neuronal death occurring in
AD [35]. It has been postulated that the reduction of Aβ generation/accumulation may be an effective therapeutic approach to block neuronal damage in AD [36,37]. Our group reported that Aβ peptide-induced hippocampal neuronal death was dose-dependently reduced in the presence of resveratrol (15–40 μM) [38,39]. Similar protective action was obtained with resveratrol and other derivatives (e.g. piceatannol) in PC12 cells [40] and murine HT22 hippocampal cell line [41]. Kwon et al. [41] reported that resveratrol inhibited both the Aβ1–42-induced activation of GSK3β and AMPK activity. Other mechanisms have also been proposed to explain the neuroprotective action of resveratrol in Aβ1-related neurotoxicity. For example, resveratrol and its derivatives were shown to directly interact with Aβ peptides by inhibiting and destabilizing the formation of Aβ1–42 fibrils [42]. However, Granzotto and Zatta [43] reported that resveratrol was unable to prevent Aβ fibril formation in human neuroblastoma cells exposed to Aβ suggesting that resveratrol acts not through anti-aggregative pathways but mainly via...
its scavenging properties. Resveratrol has also been shown to display anti-amyloidogenic effect by promoting Aβ cleavage, rather than inhibiting its production [44,45]. Indeed, using HEK293 and N2a cells transfected with human amyloid precursor protein (APP) 695, Marambaud et al. [44] have shown that resveratrol (0–40 μM) attenuated the levels of intracellular- and extracellular-secreted Aβ clearance but had no effect on the production of α, β, or γ-secretases. However, Jeon et al. [46] demonstrated that resveratrol displayed inhibitory activities of β-secretase with an IC50 of 15 μM. Moreover, resveratrol did not affect Aβ degradation by interacting with the enzymes known as metalloendopeptidase-neprilysin, endothelin-converting enzyme-1 and -2, and insulin-degrading enzyme. However, it affected proteasome through a mechanism that does not increase the total activity of the proteasome [44], which is one of the main cellular mechanisms that enzymatically degrade misprocessed and misfolded proteins [47].

It has been suggested that the accumulation of soluble forms of Aβ oligomers (known as Aβ-derived diffusible neurotoxic ligands or ADDLs), that are present in the brain and cerebrospinal fluid of AD patients [48,49], contributes to neurodegeneration occurring in AD [50]. Resveratrol has been reported to directly bind to Aβ1–42, interfere in Aβ1–42 aggregation, change the Aβ1–42 oligomer conformation and attenuate Aβ1–42 oligomeric cytotoxicity [51], whereas another group suggested that it remodels three of Aβ conformers (i.e. soluble oligomers, fibrillar intermediates, and amyloid fibrils) into aggregated forms that are non-toxic [52].

The activation of protein kinase C (PKC) may stimulate α-secretase and then non-amyloidogenic pathway in APP processing, resulting in a reduction in the production of Aβ [53]. Our group reported that dihydrochloride3-[1-[3-(dimethylamino)-propyl]-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrole-2,5-dione (GF 109203X), a PKC inhibitor, attenuated the neuroprotective effects of resveratrol against Aβ-induced toxicity in hippocampal cell cultures [38]. Moreover, Western blot data suggested that resveratrol (20–30 μM) induced the phosphorylation of the PKC delta (δ) isofrom, whose inhibition is required for initiation of the apoptotic pathway [54]. Taken together, these results support the idea that resveratrol protect neuronal cells against Aβ-induced toxicity through a mechanism that involves a stimulation of PKC isoforms (particularly the δ isofrom), possibly leading to an inactivation of GSK3β. In support of this hypothesis, phorbol 12-myristate 13-acetate, a PKC activator, has been shown to block primary hippocampal cell death exposed to Aβ1-42 and to inhibit GSK3β via a mechanism that involves serine 9 phosphorylation [55]. Finally, Vinetdeux et al. [56] studied whether AMPK – a metabolic effector that promotes neuronal survival following glucose deprivation [57] – is involved in the ability of resveratrol to lower Aβ1-42 levels and plaques. Using cultured cells, they showed that resveratrol activated AMPK by increasing intracellular calcium levels and that the inhibition of AMPK counteracted the effect of resveratrol on Aβ levels. This effect was also obtained in vivo since a peripheral administration of resveratrol activated AMPK and reduced cerebral Aβ levels and accumulation in the mouse cerebral cortex, likely via an SIRT-independent pathway [56].

5. Sirtuins

It has been reported that activation of sirtuins, particularly SIRT-1, may protect neurons against apoptosis, inflammation and oxidative stress, suggesting that these enzymes might be new targets for the treatment of neurological disorders such as stroke, AD and Parkinson’s disease (PD) [58]. Various groups showed that resveratrol is a SIRT1 activator [59–61]. Some evidence suggests that the neuroprotective action of resveratrol in models of ischemia or PD involves SIRT1. For example, Raval et al. [62] demonstrated that resveratrol can mimic ischemic preconditioning, an event that protects against subsequent ischemic insults. The inhibition of SIRT1 during reperfusion blocked the neuroprotective effect of resveratrol treatment in the hippocampus, suggesting a SIRT-dependent protective action of resveratrol. In another study, it was observed that activation of SIRT-1 induced by resveratrol strongly reduced the NF-κB signaling pathway in glial cells exposed to Aβ1-42-induced toxicity [63]. Moreover, a down-regulation of SIRT1 expression abolished the protective action of resveratrol in neuroblastoma cell line exposed to 6-OHDA [60]. More recently, Zhang et al. [64] investigated the role of SIRT1 in the neuroprotective action of resveratrol against Aβ25–35 neurotoxicity. Results showed that a treatment with Aβ25–35 reduced SIRT1 expression that was restored by resveratrol. Nicotinamide, a SIRT1 inhibitor, attenuated the protective action of resveratrol against Aβ25–35-induced toxicity. Moreover, the decrease in phosphorylation of Akt1 was associated with a down-expression of SIRT1, an effect that was reversed by resveratrol. Taken together, these findings suggest that the polyphenol activates SIRT1 which in turn enhances activity of Akt, resulting in cell protection [64]. Although resveratrol has been widely referred to as a SIRT1 activator [59,60], its ability to directly activate SIRT1 remains questionable. Recently, two groups reported that resveratrol may only indirectly increase SIRT1 activity by promoting nicotinamide adenine dinucleotide (NAD) levels induced by AMPK activation [65,66]. Moreover, resveratrol has been shown to display metabolic benefits by inhibiting αAMP phosphodiesterases in myoblast cells, leading to activation of AMPK and SIRT1 [67]. Treatment with resveratrol can improve health and survival of mice on a high-calorie diet by inducing changes associated with longer lifespan including increased AMPK activity [68]. These findings suggest that resveratrol indirectly activates SIRT1 and may be viewed as a calorie restriction mimetic with potential neuroprotective action [67,68]. Further studies are undoubtedly required to fully establish the relationship between mechanisms involved in the neuroprotective action of resveratrol and the sirtuins.

6. PI3/Akt kinases

The putative role of the phosphoinositide3-kinase (PI3-k) pathway and MAPK in the action of resveratrol was investigated in an in vitro model of ischemia [69]. The neuroprotective action of resveratrol (50 μM) in organotypic hippocampal cultures exposed to oxygen–glucose deprivation (OGD) was shown to be prevented by LY294002, an inhibitor of PI3-k, but not by PD98059, a blocker of MAPK [69]. Moreover, they showed that resveratrol induced the activation of Akt and extracellular signal-regulated kinase-1 and -2 (ERK1/2) and the phosphorylation of GSK3β, which promotes neuronal survival following glucose deprivation [57] – is required to fully establish the relationship between mechanisms involved in the neuroprotective action of resveratrol and the sirtuins.

7. Protein kinase C

Our group has shown that PKC enzymes are involved in the neuroprotective effect of resveratrol against Aβ-induced neurotoxicity [38,71]. Recently, our group has reported that the activity of the gamma (γ) isoform is linked to successful cognitive aging [72], while another group found that protein levels of PKCγ are lower in an AD mouse model [73]. It has therefore been postulated that the purported cognitive enhancing properties of polyphenols in memory-impaired animals [74] could be due to their stimulatory effects on PKCγ activity. Accordingly, we investigated the effects of resveratrol on PKCγ activation in hippocampal neuronal cells exposed to GF109203X, an inhibitor of PKC. Resveratrol prevents GF109203X-induced neuronal death and the disruption of cytoskeleton organization [75]. Interestingly, resveratrol increased both phosphorylation and expression of PKCγ, suggesting an active role for this enzyme in neuronal cell survival promoted by this polyphenol [75]. PKC signaling is involved in the regulation of multiple signaling pathways [76,77]. Future studies with novel tools such as genetically-encoded reporters, peptides derived from receptors for activated C kinase or kinase-dead mutants [for review, see [78]] will be required to evaluate the specific effect of resveratrol on PKC enzymes, notably PKCγ.
8. Programmed cell death

It has been suggested that Aβ-induced neuronal death is related to different programmed cell death (PCD) pathways; a caspase-dependent apoptotic PCD [79] and an Apoptosis Inducing Factor (AIF)-mediated, caspase-independent PCD [80]. Polyphenols may also modulate caspase-dependent and -independent programmed PCD pathways [81]. In rat cultured cortical neurons, resveratrol (0.1–10 μM) reduces apoptotic events generated by OGD/reperfusion such as increased expression of caspase-3 and -12 in neurons and the inhibition of the calcium overload [82]. More recently, it has been shown that resveratrol (50 μM) reversed apoptosis induced by OGD in primary cultured cortical neuronal cells, and this effect was accompanied by a decrease in the expression of Bax and the activation of caspase-3, as well as an inhibition of matrix metalloproteinase (MMP)-9 and -3, via a reduced activation of ERK1/2 and NF-κB pathway [83,84]. Studies investigating the role of AIF, caspase-independent PCD in the neuroprotective action of resveratrol are rather sparse. It has been reported that polyphenols including resveratrol can block in vitro glutamate and 1-methyl-4-phenylpyridinium ion (MPP+) -induced neuronal death through inhibitory action on mitochondrial AIF release [85,86]. The inhibitory action of resveratrol on AIF mitochondrial release is in agreement with previous studies of Martiniotti’s group showing that resveratrol (0.1 μM) reduced apoptotic events in dopamine-producing PC12 cells exposed to either LPS-activated microglia [87] or 1-methyl-4-phenylpyridinium ion (MPP+), a parkinsonian toxin [86]. In the latest study, Bourival et al. [86] suggest that the anti-apoptotic action of resveratrol may be due to its capacity to inhibit cytochrome c release and the significant rise of AIF (57 kDa form) in the cytosol and nucleus produced by MPP+.

9. Bioavailability and clinical efficacy of resveratrol

Resveratrol — and the preferred form trans-resveratrol — has been identified from a number of dietary sources including red grapes, berries (e.g. cranberries, bilberries, blueberries) and peanuts [5]. It is also present in red wine and to a much lesser extent in white wine. The concentration of trans-resveratrol in red wine depends on the type of red wine and its geographic origin, ranging from 0.1 to 14.3 mg/l (i.e. 0.4–57 μM) [5]. The concentrations of resveratrol (up to 50 μM) present in red wine [88,89] are much higher than those required to produce its purported in vitro protective action. Hence, only trace amounts of free resveratrol (approximately 7 μg/l i.e. 30 nM) could be detected in plasma 30 min after a 25 mg oral dose whereas the total (free and conjugated) estimated resveratrol quantity was much higher (around 450 μg/l, 2 μM) [90]. Although resveratrol has a poor bioavailability and oral administration of resveratrol undergoes an extensive metabolism in the intestine and liver [91], several animal studies have shown that it is able to cross the blood–brain barrier (BBB). For example, a chronic administration of resveratrol (8–30 mg/kg, intraperitoneal (i.p.) injections) significantly protected the hippocampus of rodents exposed to either kainic acid [92] or to an occlusion of carotid arteries [93]. In the latest study, the authors found that significant levels of resveratrol were detected in the brain after i.p. injection, reaching its peak at 4 h post-injection, supporting the hypothesis that it can cross the BBB, at least in rodents [93]. Sinha et al. [94] reported that a peripheral treatment with trans-resveratrol (20 mg/kg for 21 days) decreased the volume of infarcts in rats subjected to focal ischemia. Finally, similar in vivo protective action of resveratrol (5–300 mg/kg) was observed in transgenic models of AD [61,95]. In the latest study, injections of lipid-core nanocapsules containing resveratrol (5 mg/kg, i.p. injections) were able to rescue the deleterious effects of Aβ1–42 while treatment with resveratrol presented only partial beneficial effect [95]. According to the authors, these findings suggest that lipid-core nanocapsules strongly increased resveratrol concentration in the brain tissue [95]. It is therefore unlikely that resveratrol is the sole polyphenol that may explain the purported beneficial effects of red wine in the elderly population [3,96] since resveratrol intake of 25 mg corresponds to the consumption of more than 4 l of red wine. A few studies have been performed to assess the effects of oral resveratrol on cognitive performance in both healthy and cognitively impaired patients. Based on the vasodilatory action of resveratrol, Kennedy et al. [97] aimed to investigate whether the polyphenol improves blood flow, which thereby promotes cognitive function. In a randomized, double-blind, placebo-controlled study involving 22 healthy adults, they showed that administration of resveratrol (250 and 500 mg) resulted in dose-dependent increases in cerebral blood flow during cognitive task performance that activates the frontal cortex. However, cognitive functions were not affected. The presence of free resveratrol was confirmed by high performance liquid chromatography, with concentrations, peaking at 5.65 and 14.4 μg/l after 250 and 500 mg, respectively, 90 min after dosing [97]. In contrast, the glucuronidated and sulfated conjugates were present in much higher concentrations 45 min after the start of the cognitive tasks. Concentrations, particularly of the sulfated conjugate, continued to rise to reach about 300 and 700 μg/l for 250 and 500 mg of resveratrol, respectively, at the 90 min postdose time point that corresponded to the end of the cognitive tasks [97]. The same group reported more recently that a combination of piperine and resveratrol (250 mg) significantly augmented cerebral blood flow during task performance in the same population, as compared with placebo and resveratrol alone. However, cognitive function, mood and blood pressure were not affected whereas the plasma concentrations of resveratrol and its metabolites were not significantly different between the treatments [98]. Moreover, acute administration of resveratrol has been reported to increase both endothelium-dependent vasodilation and flow-mediated dilation of the brachial artery, an effect that may contribute to enhance cerebrovascular and cognitive functions [99]. Currently, a clinical study is under way to evaluate resveratrol as a dietary ingredient with beneficial effects in mild tomoderate AD. A phase 1, randomized placebo controlled pilot study is under way to determine whether resveratrol (250–1000 mg/day; for 12 to 52 weeks) supplementation is safe and improves memory and physical performance in older adults (ClinicalTrials.gov Identifier: NCT01126229).

10. Conclusion

Resveratrol displayed pleiotropic activities including antioxidant and anti-inflammatory effects, as well as an inhibitory action, supporting that it is one of the most promising compounds for the development of AD therapies (see Table 1). Several intracellular targets of resveratrol have also been identified. However, the poor absorption of resveratrol makes the development of analogs very challenging. The drug company Sirtris Pharmaceuticals developed a formulation of resveratrol called SRT501, with about five times higher bioavailability than resveratrol itself. However SRT501 induced multiple side effects that caused the termination of the clinical trial in patients with multiple myeloma treated. More recently, resveratrol analogs such as pterostilbene have been synthesized and have shown to be effective in rodent models of neurotoxicity [100]. Moreover, different strategies such as the use of nanotechnologies have been shown to improve the solubility and stability of polyphenols, at least in rodents. Hence various methods such as the use of lipid-core nanocapsules containing resveratrol have been shown to improve the efficacy of resveratrol. These new technologies should be used to confirm if resveratrol is a potential therapeutic agent in age-related neurodegenerative disorders.

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