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Neuroprotective Properties and Mechanisms of Resveratrol in *in Vitro* and *in Vivo* Experimental Cerebral Stroke Models

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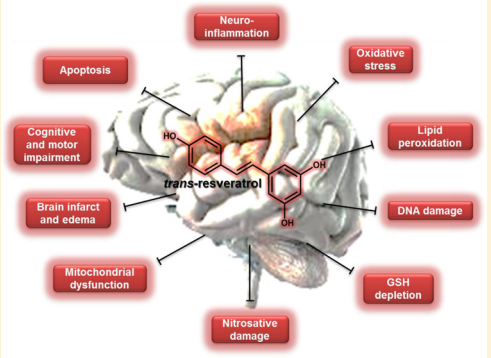
ABSTRACT: Resveratrol, a natural stilbene present at relatively high concentrations in grape skin and seeds and red wine, is known for its purported antioxidant activity in the vascular and nervous systems. In contrast to its direct antioxidant role within the central nervous system, recent research supports a protective mechanism through increasing endogenous cellular antioxidant defenses, which triggers a cascade of parallel neuroprotective pathways. A growing body of *in vitro* and *in vivo* evidence indicates that resveratrol acts through multiple pathways and reduces ischemic damage in vital organs, such as the heart and the brain, in various rodent models. Most of the protective biological actions of resveratrol have been associated with its antioxidative, anti-inflammatory, and antiapoptotic properties and other indirect pathways. Continued public interest and increasing resveratrol supplements on the market warrant a review of the available *in vitro* and *in vivo* science reported in the stroke-related literature. Rigorous clinical trials evaluating the effects of resveratrol in stroke are absent, though the general population consumption appears to be relatively safe. Resveratrol has shown potential for treating stroke in laboratory animals and *in vitro* human cell studies, yet there is still a need for human research in preclinical settings. This review summarizes many of the findings on the neuroprotective potential of resveratrol in cerebral stroke, focusing on both the *in vitro* and *in vivo* experimental models and some proposed mechanisms of action.



KEYWORDS: *Neurodegenerative disease, stilbene, neurons*

■ INTRODUCTION

An expanding body of preclinical evidence suggests that resveratrol has the potential to impact a variety of human diseases. In order to translate encouraging experimental findings into human benefits, more research is needed on the complementary nature of *in vivo* and *in vitro* studies. *In vitro* studies permit rapid screening for interactions, which are likely to be clinically meaningful, and can also be used to evaluate mechanism of action after animal studies; *in vivo* studies confirm or reject the *in vitro* prediction. The vast majority of the published studies on resveratrol performed with *in vitro* or *in vivo* models highlight its potential applications in the prevention and treatment of various disorders through multiple mechanisms of action that may be related to its health benefits.[1] Here, we review the findings on the neuroprotective potential of resveratrol from *in vitro* and *in vivo* stroke experimental models and multiple mechanisms of action that may be related to its health benefits through either direct or indirect antiapoptotic, anti-inflammatory, and antioxidative routes (Figure1). This summary also helps to clarify the relationships among *in vitro* potency with respect to mechanism of action, drug concentration, and *in vivo* efficacy in clinical and preclinical findings.

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenolic phytoalexin that occurs naturally in various edible plants.[2] Resveratrol is composed of two aromatic rings connected by a styrene double bond that allows it to exist in *trans*- and *cis*-isomers.[3,4] *trans*-Resveratrol is the preferred steric form and is recognized to have greater biologic activity if it is protected from high pH and UV light.

A popular epidemiological study suggested that the French population has a relatively low incidence of coronary heart disease, despite having a diet relatively rich in saturated fat. This phenomenon has been termed the "French paradox".[5,6] Resveratrol has been identified as a potential factor responsible for the French paradox.[7,8] In recent years, this molecule has received considerable attention for its anti-inflammatory,[9,10] antiapoptotic,[11,12] antioxidative,[13,14] antidiabetic,[13,15] antiviral,[16,17] and cardioprotective[18,19] properties.

Stroke injury initiates and activates multiple distinct but overlapping biochemical and molecular cascades, which lead to cell survival and cell damage. Figure 2 briefly describes the complex pathophysiology of stroke injury, which can be linked clinically to pathologic disturbances. Following stroke insult, there is compromised blood flow to the brain leading to reduced oxygen and ATP levels, followed by excitotoxicity and energy failure. At the same time, these cells generate free radicals, which lead to membrane, protein, and DNA damage

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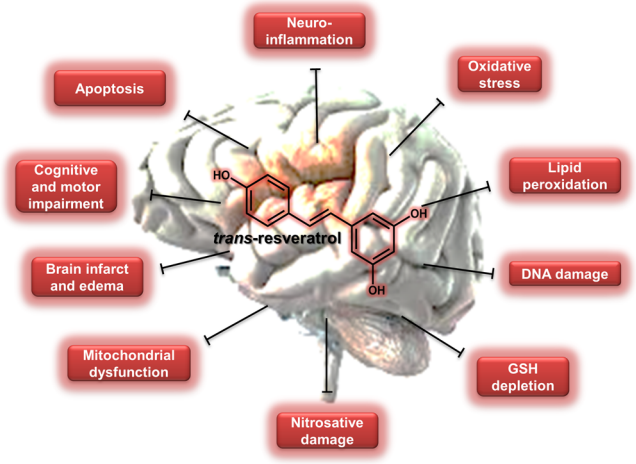

Figure 1. Potential targets associated with anti-stroke activity of resveratrol. Resveratrol exhibits therapeutic response against stroke by preventing brain infarct, edema, mitochondrial dysfunction and cognitive and motor impairment. Furthermore, it diminishes nitrosative, oxidative, and DNA damage, which leads to preclusion of apoptosis and neuroinflammation.

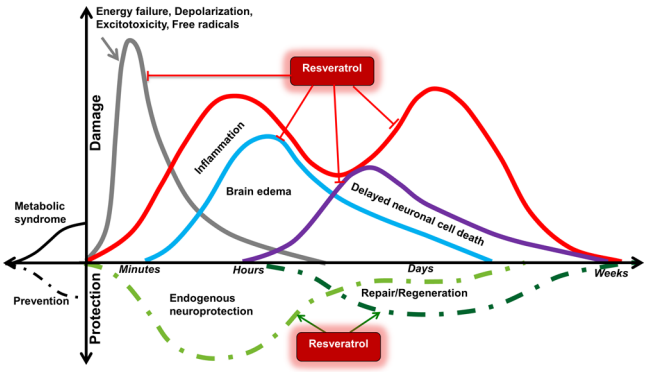

Figure 2. Schematic image showing pathophysiology of stroke-induced damage, endogenous repair, and regeneration. Adapted and modified from refs 20 and 21. Reduced blood to the brain during stroke initially affects neuronal activity and subsequently influences the metabolic and structural activity of the affected area. Several signaling cascades are activated that may be neuroprotective or detrimental to the ischemic brain. This progresses in an overlapping manner and is governed by the duration and intensity of the insult. Ischemic cascade is initiated within minutes of declined blood flow resulting in energy failure, glutamate-induced excitotoxicity, calcium overload, depolarization of the cells, and generation of free radicals. Inflammatory signaling is initiated within an hour of brain injury, and blood–brain barrier is disrupted during transition from ischemia to inflammation leading to edema and cell death. At the same time, cells initiate endogenous neuroprotective pathways to combat ischemic injury. The fate of brain cells are determined by the extent and duration of the event and the area affected by reduced blood supply. If ischemia is for longer duration, this will lead to cell death and irreversible brain damage. This review suggests that resveratrol can serve as a potential therapeutic target because it regulates and, to some extent, inhibits the detrimental pathways that are activated following injury and also up-regulates endogenous repair and regeneration processes.

and also modulate intracellular cell signaling. Thus, to re-establish and restore cellular function the most therapeutic intervention should be started as soon as possible after the stroke injury.[20,21] Neurogenesis, following stroke, has been induced using stem cells. It is a complex process, precisely regulated by undifferentiated neuronal precursor cells and differentiated neurons within the adult brain. Differentiation and proliferation of neuronal stem cells in the brain will be beneficial for injured and declining brain functions with age. A recent study has suggested that human induced pluripotent stem (iPS) cells differentiate into functional neurons and enhance functional recovery after ischemic stroke in mice.[22] Studies have also suggested that resveratrol up-regulates and modulates this differentiation and can lead to adult neurogenesis by activating the SIRT1 signaling pathway.[23,24]

BIOAVAILABILITY AND METABOLISM OF RESVERATROL

Resveratrol, a lipophilic and phenolic compound, will cross the plasma membrane and is absorbed when given orally.[25,26] It is metabolized in the body and can interact with and modulate phase I P450 enzymes CYP1A2, CYP3A4, and CYP2D6 and phase II enzymes glutathione *S*-transferase (GST) and catechol-*O*-methyltransferase (COMT).[25,26] The pharmacokinetic studies on humans and extrapolation from human cell lines suggest that 25 mg of oral resveratrol is absorbed significantly via trans-epithelial diffusion. Resveratrol has a half-life of approximately 9 h and peak active metabolite plasma concentration of approximately 2 μM.[27−29] The high and extensive metabolism of resveratrol in the intestine and liver results in approximately 1% bioavailability of the parent compound.[28,30] Interestingly, the bioavailability of resveratrol is reported to be higher during the morning hours because of circadian cycle, an important consideration for dosing schedules.[31−33] Resveratrol metabolites and polymers remain in the plasma much longer than unconverted resveratrol, whereas methylated resveratrol remains in the bloodstream for an even a longer period, a property that has been exploited in the drug development of resveratrol analogues.[34]

In vivo studies indicate that resveratrol is absorbed and distributed to a number of highly perfused tissues (i.e., liver, kidney, heart, and brain) and in the plasma, depending on the exposure time and concentration.[30,35,36] Resveratrol can also be rapidly conjugated into monosulfate and disulfate forms and can be entirely metabolized within 8 h in human hepatocyte and HepG2 cells.[37,38] Some of the most abundant metabolites of resveratrol in mammals are resveratrol-3-sulfate, resveratrol-3-*O*-glucuronide, and dihydro-resveratrol; however, they are not fully characterized.[28] Other additional metabolites have been identified and characterized as novel resveratrol-C/O-conjugated digluocuronides.[39] *In vitro* studies indicate that 50–98% of total resveratrol binds noncovalently to albumin, low-density lipoprotein, and hemoglobin.[40,41] In humans, approximately 50% of resveratrol metabolites are transported in plasma bound proteins.[39] Kidneys are the dominant excretion pathway with urinary and fecal recovery of total resveratrol between 70% and 98% within 24 h.[42,43]

Resveratrol has been studied in 40 healthy subjects from single to 29 repeated doses, and results indicate that resveratrol is quite tolerable with mild side effects of nausea and headache.[44,45] Occasionally, moderate diarrhea was also reported at higher doses compared with placebo.[44,46] Thus, because resveratrol is a pleiotropic polyphenol *in vivo*, it is likely that some of the activities that contribute to its native state will be because of its metabolites with the limitation of low bioavailability and rapid metabolism.

Table 1. *In Vitro* Examples of Resveratrol Neuroprotection

in vitro model	resveratrol treatment	stress/exposure[a]	study outcomes	ref
mice primary neuronal cultures	1, 3, or 30 μM post-treatment for 24 h	OGD for 3 h	neuroprotection	50
rat primary neuronal cultures	0.1, 1.0, or 10.0 μM pretreatment for 24 h	OGD for 2 h	antiapoptotic	87
rat cortical mixed glial cells	5, 10, 25, 50, or 100 μM post-treatment for 16 h	OGD for 0.5, 2, or 4 h	neuroprotection and anti-inflammation	104
rat endothelial cultures	100 nM to 10 μM pretreatment for 3 days	OGD for 3.5 h	antioxidation	36
HT22 cells	10 μM cotreatment for 24 h	4 mM glutamate for 24 h	direct and indirect antioxidation	55
PC12 cells	25 μM pretreatment	50 μM DTPA−Fe^{2+} and 1 mM *t*-BuOOH for 2 h	antioxidation and antimutagenic	51
	15 μM pretreatment for 6 h	H_2O_2 for 12 h	antioxidation, anti-inflammation, anticarcinogenic	62
	5, 10, or 25 μM pre- or post-treatment for 24 h	OGD for 6 h	neuroprotection	49
PC12 cells cocultured with N9 microglia	100 μM pretreatment for 3 h	LPS 1 μg/mL for 24 h	antiapoptotic	107
organotypic hippocampal slice cultures	10, 25, or 50 μM pretreatment for 24 h	OGD for 1 h	neuroprotection	47
neonatal rat midbrain slice cultures	10−100 μM	sodium azide, thrombin or *N*-methyl-*N*-nitroguanidine	neuroprotection	52
rat hippocampal slice cultures	100 μM for 20 min	OGD for 20 min	antioxidation	48

[a]DTPA, diethylenetriaminepentaacetic acid; H_2O_2, hydrogen peroxide; LPS, lipopolysaccharide; OGD, oxygen−glucose deprivation; *t*-BuOOH, *tert*-butyl hydroperoxide.

EVIDENCE OF RESVERATROL NEUROPROTECTIVE PROPERTIES: *IN VITRO* MODELS OF STROKE

Resveratrol was shown to provide protection from hypoxic and toxic insults in *ex vivo* endothelial and primary neuronal cultures (Table 1). For example, cellular damage induced by prolonged hypoxia in hippocampal slice cultures or endothelial cells was effectively attenuated by resveratrol.[47,48] Oxygen and glucose deprivation (OGD) is an *in vitro* model of hypoxia−ischemia.[49] In hippocampal slice cultures derived from 6−8 day old pups, 50 μM resveratrol has been shown to reduce OGD-induced cell death due to the activation of phosphatidylinositol 3′-kinase/Akt (PI3K/Akt pathways). This mechanism of resveratrol induced neuroprotection was delineated with LY294002, a PI3K inhibitor, but not by mitogen activated protein kinase inhibitor.[47] The stimulation of Akt and extracellular signal regulated kinase-1 and -2 (ERK1/2) and inactivation of glycogen synthase kinase-3β (GSK-3β) were also evident with resveratrol treatment.[47,48] Another study has shown that murine primary neurons exposed to OGD recovered optimal histone H3 acetylation with 30 μM resveratrol treatment.[50] Resveratrol at 25 μM concentration effectively protected rat pheochromocytoma derived (PC12) cells from the pro-oxidant properties of diethylene triamine pentaacetic acid-iron(II) (DTPA-Fe^{2+}) and *tert*-butyl hydroperoxide (*t*-BuOOH) induced cell death.[51] In midbrain slice cultures from neonatal rat, resveratrol (10−100 μM) prevented dopaminergic neuronal cell death from 1-methyl-4-phenylpyridium, sodium azide, thrombin or *N*-methyl-*N*′-nitroguanidine induced neurotoxicity. This resveratrol-mediated neuroprotection was due to reduced reactive oxygen species (ROS) and a significant increase in cellular glutathione levels.[52,53]

As low as 5 μM of resveratrol has been shown to protect the neuroblastoma cell line SH-SY5Y against the cytotoxicity caused by 300 and 500 μM dopamine.[54] Further, cell death due to kainate/glutamate toxicity in cortical and hippocampal neurons was abolished by pretreatment with resveratrol.[55,56] *N*-Methyl-D-aspartate (NMDA) mimics the action of glutamate on the NMDA receptor and the accompanying cell death after exposure, and pretreatment with resveratrol has been shown to attenuate the toxicity of NMDA exposure.[57] Similarly, primary cortical neuron cultures treated with 5−100 μM resveratrol showed protection against NMDA-induced neuronal cell death by inhibiting the elevation of intracellular calcium and ROS.[48,57] Resveratrol protected the hippocampal neuronal HT22 cell line, subjected to oxidative stress and neurotoxicity by high exposure to glutamate, by reducing the mitochondrial oxidative stress and damage through induction of the expression of mitochondrial superoxide dismutase 2 (SOD2).[55] This induction of SOD2 was mediated through the activation of PI3K/Akt and GSK-3β/β-catenin signaling pathways. Resveratrol alone (5−100 μM) increased the expression of heme oxygenase 1 (HO1) in neuron cultures, suggesting that induction of HO1 could be one of the cellular mechanisms responsible for neuroprotection.[58] In HT22 neuronal cells, reduced glutamate-induced cytotoxicity and increased HO1 expression was observed with 5, 10, and 20 μM resveratrol treatment in a concentration-dependent manner. This cytoprotection afforded by resveratrol was partially reversed by the specific inhibition of HO1 expression by HO1 small interfering RNA.[59] This suggests that the cytoprotective effect of resveratrol was at least in part associated with HO1 expression in HT22 neuronal cells. Similarly, a recent study by same group has published the cytoprotection by piceatannol (3,5,4′,3′-*trans*-trihydroxystilbene), one of the resveratrol metabolites, through HO1 in HT22 cells from glutamate-induced oxidative stress.[60] One likely pathway that we propose in promoting neuronal survival involves increased activity of HO1 through activation of the transcriptional factor Nrf2 (originally named nuclear factor erythroid 2-related factor 2).[61,62]

Table 1 summarizes the *in vitro* examples of resveratrol neuroprotection. These *in vitro* studies add to our understanding of the possible mechanisms of resveratrol-mediated neuroprotection against various neurotoxins through antioxidant and anti-inflammatory properties.

Table 2. *In Vivo* Examples of Resveratrol Neuroprotection[a]

in vivo model	resveratrol treatment (route)	dosing schedule	study outcomes	ref
neonatal mice (H–I)	0.002, 0.02, or 0.2 mg/kg (ip)	3 different time points: 24 h before H–I; 10 min before H–I; 3 h after H–I	reduced tissue loss	74
mouse MCAO	50 mg/kg (iv)	daily for 7 days	neuroprotection	70
	20 mg/kg (po)	acute, 2 h before stroke; chronic, daily for 7 days	neuroprotection from free-radical or excitotoxicity damage	61
	50 mg/kg (po)	daily for 7 days before stroke	reduced neuronal injury	69
	0.068, 0.68, or 6.8 mg/kg; (ip)	single dose after stroke	neuroprotection with extended therapeutic window	50
	1, 2.5, or 5 mg/kg (iv)	3 or 6 h after stroke	neuroprotection and anti-inflammation	78
	20 mg/kg (po)	for 3 days after stroke	neuroprotection	106
rat MCAO	30 mg/kg (ip)	first dose 3 h post-stroke and subsequent daily dose for 4 days	neuroprotection and antiapoptotic	94
	30 mg/kg (ip)	pretreated for 7 days	neuroprotection	146
	0.001 or 0.0001 mg/kg (iv)	single dose 15 min before ischemia	vasodilation, antioxidation	138
	0.001 or 0.0001 mg/kg (iv)	single dose at reperfusion	antioxidation	141
	0.1 mg/kg (iv)	twice, 15 min preocclusion and at the time of reperfusion (2 h postocclusion)	reduced infarct volume and edema, improved neurological deficits, anatomical and functional preservation	63
	15 or 30 mg/kg (ip)	pretreated for 7 days and 30 min before MCAO	reduced neurological score, infarct volume, and brain water content	66
	20 mg/kg (ip)	pretreated for 21 days	decreased infarct volume and antioxidation	71
	oxy-resveratrol, 10 or 20 mg/kg (ip)	twice, immediately after MCAO and at reperfusion	reduced infarct volume, improved neurological deficits	64
rat SAH	10 mg/kg (iv)	daily for 3 days after hemorrhage	neuroprotection, antioxidation and vasodilation	77
rat global ischemia	10, 50, or 100 mg/kg (ip)	pretreated for 48 h	neuroprotection	67
rat recurrent ischemic stroke	25 mg/kg (po)	three doses of resveratrol given daily after stroke	neuro- and cardio-protection	36
gerbil BCCAO	30 mg/kg (ip)	twice, during and shortly after CCA occlusion	decreased delayed neuronal cell death and glial cell activation, can cross blood–brain barrier	68

[a]ip, intraperitoneally; iv, intravenously; po, orally; BCCAO, bilateral common carotid artery occlusion; H–I, hypoxia–ischemia; MCAO, middle cerebral artery occlusion; SAH, subarachnoid hemorrhage.

EVIDENCE OF RESVERATROL NEUROPROTECTIVE PROPERTIES: *IN VIVO* MODELS OF STROKE

The promising neuroprotective properties of resveratrol, as shown by initial *in vitro* studies, has been validated by some *in vivo* studies. The experimental characteristics (stroke model, route of administration, dosage, time of administration, etc.) and neuroprotective outcome of resveratrol for individual *in vivo* studies have been outlined in Table 2.

In rodent stroke models, pre-, post- and delayed post-treatment with resveratrol showed neuroprotective effects as indicated by reduced infarct volume and brain water content.[63−66] Temporary bilateral common carotid artery occlusion (BCCAO) in rats results in a global ischemia in which neurons of the CA1 area of the hippocampus are selectively degenerated. Treatment with 10−100 mg/kg of resveratrol before BCCAO protected neurons in the CA1 region through the putative induction of SIRT1 activation.[67] Similarly, resveratrol (30 mg/kg during or shortly after BCCAO) treatment in gerbils showed neuroprotection by attenuating brain damage and improved cognitive outcome.[68] Further, pretreatment with 50 mg/kg of resveratrol for 7 days was shown to be neuroprotective and significantly reduced the infarct area in mice.[69,70] Resveratrol, 20 mg/kg administered via intraperitoneal,[71] intravenous,[63] and oral[61] routes during and following ischemic injury were found to inhibit the insult-induced brain damage in rats. There is also evidence that resveratrol may improve behavioral and cognitive performance in neonatal and adult neurological disorders.[72−74] The administration of resveratrol to rats was associated with improved histological, motor, and cognitive functions as measured by scoring the postural reflex, forelimb placing, corner test, foot-fault test, and performance in the Morris water maze.[75,76] Intracerebral and subarachnoid hemorrhages represent other forms of stroke in which 10 mg/kg of resveratrol treatment has also shown neuroprotection during pre- and post-treatment.[77] The most effective treatment regimen for both adult and neonatal animal models includes resveratrol administration during or immediately after the insult, although the extent of neuroprotection seems smaller after delayed administration.[72,78] In neonatal models of cerebral injury chronic treatment with resveratrol for 21 days showed less motor impairment and significantly reduced infarct volume.[71,74] Resveratrol, given prior to or after an insult in neonatal mice and rats, reduced the infarct volume after permanent and temporary middle cerebral artery occlusion (MCAO). Neuroprotection by resveratrol persisted when brain infarcts were assessed at 4, 6, and 10 weeks after MCA occlusion.[61,71,78]

In the majority of studies, brain damage was measured only for 1−7 days after insult. Research involving extended measures for the neuroprotective effect of resveratrol administration is warranted because brain injuries such as hypoxic–ischemic insult are known to evolve over a period of 6−12 weeks and possibly longer.[79] Delayed outcome of early treatment of 90 mg/kg of resveratrol in an experimental model of hypoxic–ischemic encephalopathy was investigated and confirms that resveratrol-treated animals performed better than control

groups, and there was a significant reduction in infarct and preservation of myelination.[72]

Positive outcomes of resveratrol were found to be dependent on gender and dietary nutrients.[80,81] After cerebral injury, both sexes showed reduced cerebral infarct volume and improved neurological outcomes with 5 mg/kg of resveratrol, but the protection was more prominent in females.[78,80] These findings could suggest that neonatal females are sensitive to caspase-mediated cell death pathways, because its inhibition significantly reduces injury after hypoxic ischemic insult.[82] One possible hypothesis that has been suggested for resveratrol behaving differently is that there is a similar structure to synthetic estrogen or down regulating oxidative stress response genes. Liberto and colleagues showed that resveratrol significantly increased the dopamine transporter in female striatum and not males and concluded that this difference is because resveratrol has similar chemical structure to synthetic estrogen diethylstilbestrol.[83]

NEUROPROTECTIVE EFFECTS OF RESVERATROL VIA ANTI-APOPTOSIS PATHWAY

Ischemic attack leads to oxidative stress through ROS generation, glutamate toxicity, and depletion of the intracellular antioxidant enzyme system (Figure 2). In addition to oxidative stress mediated cellular death, necrosis (energy depletion) and apoptosis (programmed cell death) are important in ischemic insult. *In vitro* and *in vivo* studies established that various cellular or biochemical pathways are involved in mitochondrial-mediated apoptotic signaling. Following stroke, cell death in male mice is triggered by poly-ADP-ribose polymerase activation and nuclear translocation of apoptosis-inducing factor[84,85] leading to caspase-independent apoptosis, and in females there is an early release of cytochrome *c* and increased caspase activation.[86]

Antiapoptotic Properties of Resveratrol *in Vitro*. OGD/reperfusion-induced apoptosis in primary neuronal cultures and resveratrol pretreatment (0.1, 1, and 10 μM) reduced cell death. In this study, resveratrol was found to prevent the overexpression of caspase-3 and caspase-12 mRNA in a concentration-dependent manner.[87] Resveratrol treatment reduces caspase-3 activation in neonatal mouse brain hypoxic ischemic injury and thus promotes cell survival.[88] In the neuroblastoma SH-SY5Y cell line, excess dopamine-induced cell death was inhibited by 50 μM resveratrol through ameliorating intracellular oxidative stress and enhancing the activity of prosurvival gene Bcl-2, thus damping the apoptotic pathways.[89] Recently, we have shown that OGD significantly up-regulated caspase-3 expression in PC12 cells and pre-, post-, and whole treatment with 25 μM resveratrol was protective and reduced caspase-3 levels.[90] Cell death following OGD and reperfusion is due to cytochrome *c* released from mitochondria and activation of caspase-3, decreased mitochondrial membrane potential, and an increased levels of pro-apoptotic gene Bax. Resveratrol at 1−30 μM prevented cell death in dopaminergic neurons, fibroblasts, human periodontal ligament cells, and PC12 cells by inhibiting ROS production, caspase-3, and Bax activities and by up-regulating Bcl-2.[53] In addition, administration of 50 μM resveratrol attenuates apoptosis by up-regulating Bcl-2 and suppresses Bax and caspase-3 in OGD and reoxygenation models. *In vitro* hypoxia reoxygenation studies showed that resveratrol prevents alteration of mitochondrial function in a concentration-dependent manner by maintaining respiratory control and ROS generation as evident by reduction of cytochrome *c* release and membrane potential collapse.[91] In summary, resveratrol has been found to regulate the expression of Bax, Bcl-2, and caspase-3 proteins in mitochondria and suppress the mitochondrial death pathway in *in vitro* hypoxia/OGD models.

Antiapoptotic Properties of Resveratrol *in Vivo*. Resveratrol treatment reduces caspase-3 activation in rats subjected to transient middle cerebral artery occlusion leading to increased cell survival.[64] It has been shown that lower doses of resveratrol (2.5 or 5 mg/kg) mediate survival signals by up-regulating antiapoptotic and prosurvival Akt and Bcl-2 gene in rats subjected to myocardial ischemia.[92] Resveratrol treatment has been shown to restore the disrupted mitochondrial integrity after cerebral ischemic damage[63] and increase the activity of mitochondrial complex I−IV and ATP production in rats. Additional findings show a decrease in cytochrome *c* release and DNA damage with improved anatomical and functional restoration of the cellular components that make up the neurovasculature of the brain. Following transient global ischemia in gerbils, there is an increase in the level of Bcl-2 associated X protein and Bax after 6 h.[93] A recent study suggested that 30 mg/kg of resveratrol reduces the ischemia reperfusion induced damage in a rat model of pMCAO. Reduced injury was due to attenuation of apoptosis and suggests that this prevention was attributed to up-regulating Bcl-2 and down-regulating Bax in the hippocampus.[94] In summary, these *in vivo* studies indicate that resveratrol exerts neuroprotection partially through modulating cell death pathways affected at large by pro-inflammatory mediators (Figures 1 and 2).

NEUROPROTECTIVE EFFECT OF RESVERATROL VIA AN ANTI-INFLAMMATORY PATHWAY

Neuroinflammation is an important contributor to the pathogenesis of neurological disorders (Figure 2). The hallmark of brain neuroinflammation is the activation of microglia.[95,96] Microglia, the resident immune cells in the brain, serve as the first line of defense when injury or disease occurs and plays a homeostatic role in the central nervous system.[97] Upon activation, microglia are capable of secreting a range of proinflammatory factors including cytokines, chemokines, ROS, reactive nitrogen species, and prostaglandins. The accumulation of these factors contributes to neuronal damage, and subsequently, the damaged neurons release debris and soluble factors, which in turn induce microglial activation (microgliosis).[98] Taken together, the inhibition of microglial activation may become a promising target for the treatment of inflammation-mediated neurological disorders. Here, we summarize the anti-inflammatory activities of resveratrol from both *in vivo* and *in vitro* studies, and highlight the inhibition of activated microglia, which may serve as a potential mechanism of neuroprotection.

Anti-inflammatory Properties of Resveratrol *in Vitro*. Neuroprotective effects of resveratrol can be seen not only in cultured neurons but also in primary microglia cultures.[99−101] This is supported by data from primary microglia cultures derived from newborn rat brain and challenged with lipopolysaccharides (LPS). Resveratrol (up to 50 μM) effectively inhibited the production of prostaglandin E_2 (PGE_2) and 8-iso-prostaglandin $F_{2\alpha}$ and suppressed the expression of cyclooxygenase-1 and microsomal prostaglandin E synthase-1.[99] Additionally, 10 μg/mL resveratrol was also found to reduce LPS-induced nitric oxide production in

primary microglia.[101] In primary mixed-glial cultures derived from newborn rat brain, 25−100 μM resveratrol provided neuroprotection via its free radical and ROS-scavenging capacity.[100] By inhibition of nuclear factor NF-κB activation, resveratrol (up to 20 μM) significantly reduced LPS-induced release of nitric oxide (NO) and PGE_2 in C6 astroglioma cells.[102] Using the N9 microglial cell line, several studies indicated that resveratrol attenuated LPS-induced phosphorylation of p38 MAPK and degradation of IκB-α, thus reducing the production of NO and tumor necrosis factor α (TNFα).[100,101] Additionally, neuronal-like PC12 cells and N9 microglia cells were cocultured to study the effect of resveratrol on LPS-induced neurotoxicity. Results showed that resveratrol could prevent the apoptosis of dopamine-producing neurons by inhibiting the production of microglia-derived TNFα and IL-1β.[103] Further, 25 or 50 μM resveratrol has been shown to suppress IL-6 gene expression and protein secretion in mixed glial cultures under hypoxia/hypoglycemia conditions.[104]

Anti-inflammatory Properties of Resveratrol *in Vivo*. Attenuation of microglial activation is a therapeutic strategy in ischemic stroke.[105] Administration of 10−40 mg/kg of resveratrol 3 h after MCAO effectively reduced the increased expression of the pro-inflammatory cytokines IL-1β and TNFα,[105] and this effect lasted for up to 24 h after reperfusion. The neuroprotective effect of resveratrol requires peroxisome proliferator activated receptor-α expression, which may exert anti-inflammatory effects by antagonizing NF-kB.[106] Others have reported that increases in IL-1β, TNFα, and nitric oxide production associated with NF-κB signaling and phosphorylation of p38 from activated microglia are inhibited by resveratrol.[101,107] Resveratrol suppressed expression of IL-1β and TNFα, microglial activation, and ROS production in the ischemic cortex.[78] In cerebral ischemia produced by occlusion of both common carotid arteries in gerbils, 30 mg/kg of resveratrol treatment during ischemia and 24 h after ischemia significantly prevented neuronal cell death and inhibited glial cell activation.[68]

Resveratrol administration 3 h after insult during the acute phase of ischemic stroke reduces brain injury in both male and female mice at different doses (5 mg/kg for males and 1 mg/kg for females).[78] Administration of resveratrol 6 h after insult was also effective in decreasing infarct volumes.[78] These findings suggest that suppression of inflammation is associated with the neuroprotective effects of resveratrol, and resveratrol can be developed as a therapeutic drug for stroke treatment (Figure 1). Furthermore, decreased neuroinflammatory properties of resveratrol can be demonstrated by three mechanisms: (1) inhibition of ROS production; (2) suppression of MAPK signal transduction pathways; and (3) activation of the SIRT1 pathways, which in turn could suppress the activation of the NF-κB signaling pathways. The overall effects of these events lead to reduced pro-inflammatory mediators, eventually producing neuroprotection.

NEUROPROTECTIVE EFFECT OF RESVERATROL VIA ANTIOXIDATIVE PATHWAY

Oxidative stress plays an important role in the appearance and development of neurodegenerative disorders and a number of protective enzymes including superoxide dismutase, catalase, glutathione peroxidase, and heme oxygenase essentially contribute to cellular defense in the brain working against oxidative stress.[108] Resveratrol is reported to be beneficial in neurological disorders by attenuating this oxidative stress (Figure 1). The beneficial effects are thought to be due to its antioxidative properties. Resveratrol attenuates oxidative stress by directly scavenging free radicals and indirectly increasing endogenous cellular antioxidant defenses, for example, via activation of the Keap1−Nrf2 pathway (Figure 2). The brain has lower antioxidant defenses than other organs such as the liver and kidney;[109] however, Nrf2 is expressed throughout the brain[110] and plays a key role in the cellular defenses against oxidative stress by regulating the expression of inflammatory markers and antioxidant enzymes.[111,112] Nrf2 also modulates microglial dynamics[113] and protects neurons and astrocytes from toxic insults.[114,115] A study in oligemia model of stress/stroke using immunohistochemistry showed localization of Nrf2 in neurons of the cingulate cortex and cerebellar Purkinje cells.[116] Srivastava and colleagues reported the first quantitative measurements of temporal and spatial Nrf2 distribution in the brain of rats following stroke by using a novel immunohistochemical technique.[117]

Normally, under basal conditions, in the absence of cellular stress, the nuclear content of Nrf2 is small and is largely bound to Keap1 within the cytoplasm, which anchors and limits Nrf2-mediated gene expression. Under conditions of chemical/oxidative stress, the Keap1−Nrf2 complex is dissociated, and Nrf2 is able to evade Keap1, accumulate within the nucleus, and trigger antioxidant response element (ARE)-mediated gene expression and transcription of phase II defense enzymes and antioxidant stress proteins such as NAD(P)H quinone oxidoreductase 1 (NQO1), γ-glutamylcysteine synthetase, HO1, glutathione (GSH)−peroxidase, GSH-S-transferase, and superoxide dismutase to attenuate oxidative stress. A study using Nrf2 knockout ($Nrf2^{-/-}$) mice has confirmed that this mouse strain has a lower expression of such enzymes and antioxidant stress proteins, and is more susceptible to oxidative stress.[118−120]

The Nrf2/ARE pathway can be activated pharmacologically and by dietary means.[121,122] Nrf2 seems to autoregulate its own expression through weak ARE-like cis-elements in its promoter,[123] which leads to persistent accumulation of Nrf2 in the nucleus and protracted induction of protective genes in response to inducers. Resveratrol increases the transcriptional activity of Nrf2 and up-regulates several ARE-regulated genes involved in free radical metabolism in an Nrf2-dependent manner.

Resveratrol was found to be a potential scavenger of superoxide, hydroxyl, and metal-induced radicals, as well as to show antioxidant abilities in cells producing ROS, at least *in vitro*. It is protective against lipid peroxidation in cell membranes and DNA damage caused by ROS (Figure 1). Studies have suggested that continued dietary resveratrol would also protect against aging and delay age-related cognitive decline. This finding was linked to the activation of multiple pathways via elevating cAMP levels, peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) activity, and SIRT1 as previously discussed.[124,125] Although resveratrol seems to attenuate oxidative insult,[126] a new finding showed that LPS-induced activation of microglial NADPH oxidase and consequent production of superoxide was inhibited by resveratrol.[127]

Antioxidative Properties of Resveratrol *in Vitro*. Resveratrol exhibits neuroprotection likely by its indirect neuroprotective effect via activation of Nrf2/ARE pathways. Resveratrol augmented cellular antioxidant defense through HO1 induction via Nrf2/ARE signaling in PC12 cells[62] and in

primary neuronal cultures[128] from oxidative stress and also protected cultured hippocampal neurons against nitric oxide-mediated cell death.[129] Nrf2-dependent induction of major cellular antioxidant enzymes effectively attenuate cellular and mitochondrial oxidative stress in cultured endothelial cells.[130,131] Furthermore, resveratrol (50 or 100 μM) exerts beneficial neuroprotective effects through inducing mitochondrial manganese superoxide dismutase (MnSOD) expression and activity in the human fetal lung fibroblast MRC-5 cell line[132] and up-regulates genes involved in antioxidant pathways.[133] Resveratrol administration attenuates free radical formation and increases glutathione content and SOD in PC12 cells receiving OGD and reoxygenation.[90] Similarly, 10 μM resveratrol attenuated paraquat-induced ROS production and strongly activated the Nrf2 signaling pathway to further induce ARE-dependent cytoprotective genes against the toxicity and oxidative stress in human bronchial epithelial BEAS-2B cells.[134]

Antioxidative Properties of Resveratrol *in Vivo*. Several studies have shown that 20−50 μM resveratrol activates the Nrf2/ARE pathway in different experimental *in vivo* models.[66,135,136] Previous findings from our laboratory showed that pretreatment with resveratrol attenuates ischemic injury, and this effect was obliterated in HO1 knockout mice.[61] Similar findings from other groups showed that resveratrol pretreatment for 7 days significantly attenuated neurological deficits, infarct volume, and edema. It also reduced the oxidative stress as indicated by the levels of MDA and SOD and up-regulated the expression of transcription factor Nrf2 and HO1 after focal cerebral ischemia/reperfusion injury in rats[66] and in PC12 cells.[137] Huang et al. showed that 15 min pretreatment with resveratrol reduces the infarct size following focal cerebral ischemia in rats.[138] A similar effect was shown in which mice pretreated with 20 mg/kg of resveratrol had decreased motor impairment and infarct size and a rise in lipid peroxidation with reduced glutathione levels.[71] Further studies suggest that resveratrol administration suppressed free radical production in the ischemic cortex of mice[139] and also restored the levels of mitochondrial glutathione and glucose 6-phosphate dehydrogenase with a significant decrease in mitochondrial lipid peroxidation in a MCAO model of brain ischemia. This protection by resveratrol could be attributed to its properties of reactive oxygen/nitrogen species scavenging[63] and augmentation of antioxidant enzymes.[136] Similarly, Shin and colleagues[78] showed that resveratrol administration reduces free radical burst and microglial activation. Dietary resveratrol (1 mg/kg, oral for 8 weeks) reduced oxidative DNA and glycoxidative stress in hypertensive male and female rats.[80] Likewise, Ungvari and colleagues showed that resveratrol attenuates mitochondrial and cellular oxidative stress induced by hyperglycemia in endothelial cell cultures, and this was reversed by small interfering RNA mediated knockdown of Nrf2 or overexpression of Keap1.[140] Resveratrol also reduces nitrosative damage following ischemia reperfusion injury (Figure 1) via up-regulating the levels of NO in plasma and endothelial nitric oxide synthase (eNOS) and down-regulating inducible nitric oxide synthase (iNOS).[141] These studies provide strong evidence that resveratrol could exert a neuroprotective effect by modulating antioxidant enzymes and antioxidant responsive elements.

RESVERATROL IN CLINICAL TRIALS

A few human studies have been published in highly respected academic journals that have explored the potency of resveratrol to achieve physiological benefits that have been observed in laboratory models.[142,143] Most have focused on characterizing the pharmacokinetics and metabolism of resveratrol. A rapid uptake of resveratrol is observed in humans. About 30 min after a low dose intake, the plasma concentration of resveratrol peaks, although under fasting conditions, higher doses of resveratrol showed a delayed peak to 1.5 or 2 h.[28] A few epidemiological studies have suggested that moderate consumption of red wine increases overall survival rates in various populations.[142]

While resveratrol content in wine can vary greatly, wine consumption has been associated with a significant reduction in the risk for stroke and may help prevent subsequent strokes. A 12 year study from Copenhagen City Heart suggested that the patients who consumed red wine had 50% less risk of dying from coronary heart disease or stroke as compared with those who never consumed wine.[19] One main issue with red wine is that the quantity of active resveratrol varies greatly, and it is essentially impossible to know the amount consumed without rigorous analytical studies. Further, the capacity of resveratrol to alter cerebral blood flow varies in humans as found in a randomized, double-blind, placebo-controlled, crossover study.[144] A single oral dose of 500 mg of resveratrol increased cerebral blood flow in the prefrontal cortex during task performance, as indexed by total concentrations of hemoglobin in healthy young adults.

In another study, ten healthy humans were given a high-fat, high-carbohydrate meal on two occasions; one with a placebo and the other with a supplement containing 100 mg of resveratrol and 75 mg of grape skin polyphenols.[145] The resveratrol supplement and grape skin polyphenols suppressed the increase in oxidative stress, suggesting a strong antioxidant effect, and stimulated the activity of Nrf2 following the meal along with inducing the expression of the related antioxidant genes, NQO1 and glutathione S-transferase P1. These effects are observed despite extremely low bioavailability and rapid clearance of resveratrol from the circulation. There is little evidence to support physiological benefits of resveratrol in humans, and the limited data available provides a strong justification for further clinical trials.

POTENTIAL RECOMMENDATIONS AND CONCLUSIONS

Resveratrol has encouraging potential and holds great promise for future development as a therapeutic agent for neurodegenerative diseases. Given that many targets for resveratrol have been identified *in vitro* and effective doses vary over at least 3 orders of magnitude *in vivo*, combined with a relatively good safety profile, it seems likely that resveratrol acts through multiple pathways. We believe that healthy individuals may still benefit from resveratrol's multipotential to delay aging and lifestyle-induced decrements in health. Care should be taken to determine the potential beneficial effects of a conservative amount of red wine, weighed against other, perhaps non-beneficial, components in wine. *In vitro* and *in vivo* animal data are promising and highlight the need for human clinical trials, but are not currently strong enough to justify recommendations for the chronic administration of resveratrol to human beings. Future research should aim at exploring the relationship

between dose, bioavailability, efficacy, and either direct or indirect mechanisms of actions in humans and further controlled clinical trials should be conducted to determine the preventive and therapeutic efficacy of either dietary or supplemented resveratrol.

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LIST OF ABBREVIATIONS

ARE, antioxidant response element; BCCAO, bilateral common carotid artery occlusion; DTPA−Fe^{2+}, diethylene triamine pentaacetic acid−iron(II); HO1, heme oxygenase 1; LPS, lipopolysaccharides; MCAO, middle cerebral artery occlusion; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; OGD, oxygen−glucose deprivation; PC12, pheochromocytoma cells; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; SAH, subarachnoid hemorrhage; SOD2, superoxide dismutase 2; *t*-BuOOH, *tert*-butyl hydroperoxide; TNFα, tumor necrosis factor α

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