Published in final edited form as:

Neurochem Int. 2015 October ; 89: 75–82. doi:10.1016/j.neuint.2015.08.009.

Resveratrol Neuroprotection in Stroke and Traumatic CNS injury

Mary Lopez, Robert J Dempsey, and Raghu Vemuganti

Department of Neurological surgery, University of Wisconsin, Madison, WI USA

Abstract

Resveratrol, a stilbene formed in many plants in response to various stressors, elicits multiple beneficial effects in vertebrates. Particularly, resveratrol was shown to have therapeutic properties in cancer, atherosclerosis and neurodegeneration. Resveratrol-induced benefits are modulated by multiple synergistic pathways that control oxidative stress, inflammation and cell death. Despite the lack of a definitive mechanism, both in vivo and in vitro studies suggest that resveratrol can induce a neuroprotective state when administered acutely or prior to experimental injury to the CNS. In this review, we discuss the neuroprotective potential of resveratrol in stroke, traumatic brain injury and spinal cord injury, with a focus on the molecular pathways responsible for this protection.

Keywords

Polyphenols; Neuroprotection; Ischemia; CNS injury; Oxidative stress; Inflammation

1. Introduction

Resveratrol is a naturally occurring stilbene-class of polyphenol produced in the skins of many edible plants as a response to fungal infection (Siemann and Creasy, 1992; Takaoka, 1940). Resveratrol is widely known for its anti-oxidant properties, and has been implicated in the putative anti-atherosclerotic effects of red wine. The neuroprotective benefits of resveratrol were known since it was shown to ameliorate kainate-induced excitotoxicity (Virgili and Contestabile, 2000). Subsequently, resveratrol has been shown to improve histopathological and behavioral outcomes after various types of acute CNS injuries including stroke (Girbovan et al., 2012; Huang et al., 2001; Karalis et al., 2011), traumatic brain injury (TBI) (Singleton et al., 2010; Sonmez et al., 2007), subarachnoid hemorrhage (SAH) (Shao et al., 2014) and spinal cord injury (SCI) (Ates et al., 2006; Kaplan et al., 2005).

The exact mechanism of resveratrol-induced neuroprotection is not clear (Morris-Blanco et al., 2014; Park et al., 2012; Tang, 2010), but many of its beneficial effects were thought to

---

Address Correspondence to Raghu Vemuganti, PhD, Professor, Dept. of Neurological Surgery, University of Wisconsin, Madison, WI 53792, vemuganti@neurosurgery.wisc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

be promoted by activation of silent mating type information regulation 2 homolog 1 (SIRT1) (Borra et al., 2005), AMP-activated kinase (AMPK) (Dasgupta and Milbrandt, 2007) and nuclear factor (erythroid derived 2)-like 2 (Nrf2) (Chen et al., 2005; Ungvari et al., 2010). SIRT1 is a nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylase that acts on histone and non-histone targets to improve lifespan and promote a pro-survival environment in the CNS (Wood et al., 2004; Yang et al., 2013). AMPK senses increases in endogenous adenosine levels, specifically AMP or ADP, and compensates by enhancing ATP production. AMPK has been shown to activate acetyl-coA carboxylase and SIRT1, while suppressing the mammalian target of rapamycin complex (mTORC), resulting in an overall improvement in metabolism and increased lifespan (Baur et al., 2006; Spasi et al., 2009). Nrf2 is a transcription factor that is responsible for binding antioxidant response elements (ARE) in the promoters of genes like superoxide dismutase (SOD), heme oxygenase 1 (HO-1), catalase and many other phase II defense enzymes, inducing their expression (Chen et al., 2005; Kansanen et al., 2013; Zhang et al., 2013).

The secondary brain damage and neuronal death after an acute CNS insult like stroke are synergistically mediated by many pathophysiologic mechanisms that include oxidative stress, inflammation, ionic imbalance and apoptosis. Treatment with resveratrol is shown to prevent or slow-down many of these pathological changes and its neuroprotective actions seem to be mediated by many putative effectors and targets (Fig. 1). The goal of this review is to discuss the major mechanisms that are thought to mediate resveratrol-induced neuroprotection.

2. Pathophysiology of Acute Ischemic Stroke and Traumatic CNS Injury

In order to appreciate mechanisms of resveratrol-mediated neuroprotection, one must consider the pathophysiology of ischemic stroke and traumatic CNS injury. Ischemic and traumatic injuries to the CNS are very different in nature. Ischemic stroke mostly occurs when the arteries (for example, the middle cerebral artery) that supply blood to the brain are blocked, often by atherosclerotic and thrombotic processes (Dirnagl et al., 1999). TBI/SCI are physical injuries most often caused by sports, motor accidents and combat (Nolan, 2005; Silva et al., 2014). However, the molecular and cellular mechanisms that promote secondary cell death and tissue damage are strikingly similar between ischemic and traumatic injuries to CNS (Fig. 2). In both cases, the primary insult leads to unavoidable damage in the epicenter immediately after the event, while the secondary damage progresses for days and encompasses the areas surrounding the primary injury (Dirnagl et al., 1999; Masel and DeWitt, 2010). Within minutes of an acute insult, secondary injury starts with energy failure that results in shutdown of Na⁺/K⁺-ATPase leading to ionic balance that promotes edema. Excitotoxicity (increased release and decreased reuptake of glutamate leading to overactivation of ionotropic glutamate receptors) also starts within minutes of an insult to CNS. Glutamate receptor activation allows lethal amounts of Ca²⁺ to enter the cell that activate proteases, nucleases and enzymes that form free radicals which are toxic to neurons (Hazell, 2007; Obrenovitch and Urenjak, 1997). Following these events, the second line of pathophysiological events that include inflammation, oxidative/nitrosative stress and endoplasmic reticulum (ER) stress starts within hours (Lakhan et al., 2009; Nakka et al., 2014). Activation of microglia releases pro-inflammatory cytokines that attracts blood-borne

macrophages and neutrophils to extravasate into CNS. All these cells release pro-inflammatory cytokines and reactive oxygen species (ROS) and reactive nitrogen species which are highly toxic to neurons (Manzanero et al., 2013). In addition, activation of NADPH oxidase forms ROS and curtails functioning of anti-oxidant enzymes like SOD and catalase promotes oxidative stress. Energy failure also leads to ineffective folding of proteins in ER lumen leading to accumulation of unfolded/misfolded proteins that activates ER stress pathways and the downstream apoptotic transcription factors like CCAAT-enhancer-binding protein homologous protein (CHOP) and activating transcription factor 4 (ATF4). Inflammation, oxidative/nitrosative stress and ER stress are simultaneous and potentiate each other (Nakka et al., 2014). In addition to these events, recent evidence suggest that epigenetic changes, alterations in the levels and function of non-coding RNAs like microRNAs and post-translational modifications such also play a role in promoting secondary brain damage after acute CNS insults (Hochrainer et al., 2012; Lee et al., 2014; Nakka et al., 2011).

3. Anti-oxidative effects of resveratrol

Following an ischemic stroke, ROS levels increase quickly during the period of occlusion followed by a second wave upon reperfusion (Domínguez et al., 2010; Zweier and Talukder, 2006). If uncontrolled, oxidative stress kills neurons (Manzanero et al., 2013). Paradoxically pathways that promote as well as fight oxidative stress are induced after stroke, however most often the magnitude of ROS production supersedes ROS disposal. A major target of resveratrol is the anti-oxidant transcription factor Nrf2 (Chen et al., 2005; Ungvari et al., 2010). In physiological conditions, Nrf2 is bound to Kelch ECH associating protein 1 (Keap1) in the cytoplasm that prevents Nrf2 activation. High levels of oxidative stress dissociate the Nrf2-Keap1 complex, allowing Nrf2 to translocate into the nucleus and express a multitude of enzymes that induce an anti-oxidant environment (Kansanen et al., 2013; Zhang et al., 2013). This will help the system to dispose the ROS and promote protein chaperoning leading to neuroprotection. Several studies showed that resveratrol treatment promotes post-ischemic Nrf2 activation and expression of many of its down-stream genes (Kesharwani et al., 2013; Ren et al., 2011).

Many studies showed that resveratrol treatment also activates PPAR γ coactivator 1 α (PGC-1 α) which has innate properties as a free radical scavenger (Lagouge et al., 2006; Lorenz et al., 2003; Ungvari et al., 2010). PGC-1 α was shown to modulate the mitochondrial anti-oxidant enzymes SOD2, thioredoxin and glutathione peroxidase 1 (GPX1) and thus mitigates oxidative stress (Lu et al., 2010; St-Pierre et al., 2006). PGC-1 α was also thought to ameliorate oxidative burden by increasing mitochondrial function and minimizing ROS buildup via organelle biogenesis (St-Pierre et al., 2003). Interestingly, PGC-1 α activity was increased by AMPK-mediated phosphorylation (Jäger et al., 2007) and sirtuin 1 (SIRT1)-mediated deacetylation (Rodgers et al., 2005), representing two distinct modes for resveratrol-mediated activation of this regulator of oxidative metabolism. Pretreatment with resveratrol was also shown to induce PGC-1 α after ischemic stroke (Shin et al., 2012). Furthermore, the chemical structure of resveratrol allows it to directly scavenge free radicals by hydrogen atom transfer and sequential proton loss electron transfer, which

can modulate cell signaling and decrease oxidative damage in a dose-dependent manner (Shang et al., 2009).

Resveratrol-mediated decrease in neuronal MDA levels is often associated with increased levels of antioxidant enzymes such as SOD (Rege et al., 2013) and antioxidant compounds such as glutathione (GSH) (Sinha et al., 2002). In particular, the antioxidant enzyme HO-1 is implicated as a major effector of resveratrol-mediated neuroprotection after post-ischemic reperfusion (Chen et al., 2005; Zhuang et al., 2003). Resveratrol treatment induces HO-1 expression in cultured mouse cortical neurons (Zhuang et al., 2003). Furthermore, resveratrol-induced HO-1 was shown to protect against H₂O₂ exposure in PC12 cells (Chen et al., 2005). This result was later corroborated by a study of naïve and H₂O₂ exposed C6 astrocytes, in which resveratrol was shown to induce HO-1 and decrease nitrite production (Quincozes-Santos et al., 2013). A study of cultured mouse cortical neurons also demonstrated that resveratrol treatment protects against glutamate exposure, and that this protection can be abolished with an HO-1 inhibitor (Sakata et al., 2010). The same study showed that HO-1 knockout mice fail to exhibit the neuroprotection afforded by resveratrol pretreatment (either acute at 2h before ischemia or daily for 7 days) following transient focal ischemia. Resveratrol pretreatment was also shown to increase protein level and activity of Nrf2 and HO-1, which correlate with improved SOD activity and decreased lipid peroxidation, less edema, smaller infarcts, and improved neurologic function in rats subjected to focal ischemia (Ren et al., 2011). Resveratrol treatment was also shown to increase nuclear levels of the anti-oxidant transcription factor Nrf2 leading to decreased oxidative stress and reduced mitochondrial protein oxidation following spinal cord ischemia in rodents (Keshewani et al., 2013).

TBI, SCI and SAH in rodents lead to increased levels of lipid peroxidation as a result of ROS generation (Bains and Hall, 2012; Hall et al., 2010). Resveratrol treatment was shown to decrease the oxidative damage in all these 3 models (Ates et al., 2006; Ates et al., 2007; Karaoglan et al., 2008). The earliest study of oxidative stress after resveratrol treatment in traumatic CNS injury compared methylprednisolone (MP) treatment to resveratrol treatment in a weight-drop model of rat SCI, and showed that resveratrol decreased lipid peroxidation and edema, and increased ultrastructural integrity (Yang and Piao, 2003). This was supported by a subsequent study that showed that resveratrol treatment decreased MDA levels and preserved mitochondrial integrity after spinal cord ischemia in rabbits (Kaplan et al., 2005). Another study of resveratrol in rat SCI also showed increased GSH levels, decreased MDA and nitric oxide (NO) levels and xanthine oxidase (XO) activity (Ates et al., 2006). Recently, resveratrol was reported to upregulate SOD activity in a weight-drop model of rat SCI leading to decreased lipid peroxidation (Liu et al., 2011). Resveratrol exhibited anti-oxidant neuroprotective effects after TBI as well. Resveratrol treatment was shown to reduce lipid peroxidation by increasing GSH levels, decreasing NO levels, and reducing XO activity, resulting in decreased lesion size after controlled cortical impact injury in the adult rat (Ates et al., 2007). Furthermore, a study of SAH by autologous blood injection into the subarachnoid space of adult rats showed that resveratrol treatment decreases MDA levels and increases SOD activity (Karaoglan et al., 2008). The same study also showed improvement in basilar arterial lumen diameter and endothelin-1 activity after resveratrol treatment.

4. Anti-apoptotic effects of resveratrol

Resveratrol is well known for its pro-apoptotic role in cancer (Jang et al., 1997; Lin et al., 2011), but interestingly has an anti-apoptotic effect after acute CNS injury. Resveratrol treatment was shown to inhibit cytochrome c immunoreactivity and caspase-3 activity after focal ischemia in adult rodents (Andrabi et al., 2004; Ren et al., 2011), after hypoxic-ischemia in neonatal rodents (West et al., 2007) and after oxygen-glucose deprivation (OGD) in primary neuronal cultures (Gong et al., 2007). Furthermore, resveratrol treatment prevented OGD-mediated increases in hypoxia inducible factor 1- α (HIF-1 α), Bax and caspase-3, while increasing the levels of anti-apoptotic Bcl2 in PC12 cells (Agrawal et al., 2011). Resveratrol-mediated improvement of Bcl2/Bax ratio has also been demonstrated in rats subjected to focal ischemia (Li et al., 2012) and in primary cortical rat neurons subjected to OGD (Gao et al., 2014; Wang et al., 2009). Bcl2 upregulation and Bax inhibition can be caused by phosphoinositide-3 kinase (PI3K)/Akt signaling, which is shown to be activated by resveratrol treatment in the ischemic brain (Shin et al., 2012) as well as independent of ischemic stroke (Tsai et al., 2012). Furthermore, PI3K inhibition was shown to attenuate resveratrol-induced glycogen synthase kinase 3- β (GSK-3 β) activation in hippocampal slices subjected to OGD (Zamin et al., 2006). In addition, increased GSK-3 β activity after resveratrol treatment was correlated with p-Akt in a rat model of transient global ischemia (Simao et al., 2012b). A recent study of OGD in primary mouse cortical neurons showed that resveratrol neuroprotection is also mediated by RelA-specific acetylation at Bcl-XL promoter sites and decreased RelA-specific acetylation at Bim promoter sites (Lanzillotta et al., 2013). This same study also showed increased RelA binding/histone acetylation on Bcl-XL promoters and decreased RelA binding/histone acetylation on Bim in a mouse model of transient focal ischemia.

Resveratrol treatment was shown to prevent autophagic cell death by decreasing GSK-3 β activation after controlled cortical impact-induced TBI in adult rats (Lin et al., 2014). The proposed mechanism for this action of resveratrol was suggested to be prevention of ROS formation, however this was not substantiated by any demonstration of Akt-independent GSK-3 β inhibition (Lin et al., 2014). Resveratrol has also been shown to decrease apoptosis in an animal model of SAH. After deposition of autologous blood in the rat subarachnoid space, intravenous resveratrol injection was shown to increase p-Akt levels, concomitantly decreasing caspase-3 levels, and leading to prevention of neuronal apoptosis (Zhou et al., 2014). This study also showed attenuation of the anti-apoptotic effects of resveratrol with a PI3K inhibitor, further supporting the importance of the PI3K/Akt pathway in resveratrol-mediated neuroprotection. Resveratrol was also shown to have an anti-apoptotic effect by improving Bcl2/Bax ratio and preventing caspase-3 activation leading to decreased number of TUNEL-positive neurons and improved cellular ultrastructure after SCI in adult rats (Liu et al., 2011).

5. Anti-inflammatory properties of resveratrol

Uncontrolled inflammation following ischemic and traumatic injuries to CNS is known to promote secondary brain damage. Many studies showed that resveratrol treatment decreases inflammatory burden by reducing microglial activation in *in vivo* (Girbovan and Plamondon,

2015; Shin et al., 2010; Simao et al., 2012a) and *in vitro* (Song et al., 2014) models of ischemic stroke. Resveratrol has also been shown to reduce edema (Ren et al., 2011; Yousuf et al., 2009) and prevent the release of pro-inflammatory cytokines (Shin et al., 2010; Song et al., 2014). Furthermore, resveratrol alleviates inflammation by decreasing ROS levels which are known to activate immune cells (Khodr and Khalil, 2001). It is interesting that resveratrol and several other trans-stilbenes inhibit NF- κ B signaling independently of their antioxidant capacity (Heynekamp et al., 2006). In primary cortical neurons, resveratrol treatment was shown to activate SIRT1, inhibiting nuclear translocation of NF- κ B subunit p65 following OGD (Wang et al., 2009). This data is also supported by empirical evidence that SIRT1 inhibits NF- κ B activity (Yeung et al., 2004). NF- κ B can be inhibited by peroxisome proliferator-activated receptor (PPAR) isoforms as well (Delerive et al., 2000) and resveratrol was shown to activate both PPAR γ and PPAR α in primary cortical neurons (Calleri et al., 2014). Furthermore, post-ischemic neuroprotection induced by resveratrol was attenuated in PPAR α knockout mice (Inoue, 2003). Hence, the actions of resveratrol in attenuating NF- κ B signaling might be mediated by both SIRT1 and PPAR. Resveratrol treatment was also shown to inhibit the expression of the pro-inflammatory cytokine interleukin-6 (IL-6) after hypoxia/hypoglycemia in a primary glial cell culture (Wang et al., 2001). Furthermore, resveratrol has been shown to prevent TNF α signaling through its action on NF- κ B after lipopolysaccharide (LPS) exposure in an N9 microglial cell line co-cultured with primary rat microglia (Bi et al., 2005). A similar study of N9 microglia co-cultured with PC12 cells also showed that resveratrol and the nutraceutical quercetin could prevent LPS-induced TNF α and interleukin-1 α (IL-1 α) (Bureau et al., 2008). These studies demonstrate that resveratrol treatment could attenuate pro-inflammatory cytokine release by microglia, preventing apoptosis and thus promoting cell viability. Another study showed that in BV2 microglia subjected to hypoxia, resveratrol treatment decreased TNF α and NF- κ B nuclear translocation with concomitantly increased levels of the anti-inflammatory cytokine interleukin-10 (IL-10) and brain derived neurotrophic factor (BDNF) that improve cell viability (Song et al., 2014). Thus, resveratrol can influence both pro- and anti-inflammatory cytokines that play key roles in NF- κ B regulation. Resveratrol treatment was also shown to inhibit TNF α and interleukin-1 β (IL-1 β) in following ischemic stroke in adult mice (Shin et al., 2010). Another potent anti-inflammatory action of resveratrol is its ability to inhibit cyclooxygenase-1 and -2 (COX-1 and COX-2) leading to reduced pro-inflammatory arachadonic acid (AA) metabolism (Mohamed et al., 2014; Simao et al., 2012a). In rat microglial cultures, resveratrol treatment was shown to inhibit LPS-induced microsomal prostaglandin E synthase-1 (mPGES-1) and the concomitant formation prostaglandin E2 (PGE $_2$), and ROS-induced 8-iso-prostaglandin F $_{2\alpha}$ (8-iso-PG F $_{2\alpha}$) indicating its further anti-inflammatory potential (Candelario-Jalil et al., 2007). Resveratrol treatment was shown to attenuate neutrophil infiltration in a rabbit model of spinal cord ischemia (Kaplan et al., 2005).

Resveratrol was also shown to attenuate inflammation after TBI, SAH and SCI. A recent study showed that resveratrol treatment decreased microglial activation and levels of the pro-inflammatory cytokines IL-6 and IL-12 following mild TBI in mice (Gatson et al., 2013). Resveratrol treatment following SAH was shown to prevent p65 nuclear translocation attenuating NF- κ B signaling, and preventing transcription of TNF α , IL-1 β , IL-6, and matrix

metalloproteinase-9 (MMP-9) leading to decreased edema and less BBB disruption (Shao et al., 2014). Resveratrol treatment was shown to decrease edema and inflammatory cytokine release (specifically IL-1 β , IL-10, TNF α , and myeloperoxidase) after SCI in rats (Liu et al., 2011). Clinical studies showed that SCI patients suffer sub-lesion bone loss (Kocina, 1997), and resveratrol treatment prevents IL-6 and MDA levels leading to increased genesis of osteoblasts and osteoclasts in an experimental rat model of SCI (Wang et al., 2013).

6. Resveratrol treatment mimics ischemic preconditioning

Ischemic preconditioning (IPC) is a phenomenon wherein a sub-lethal ischemic insult prepares the organ for a more severe ischemic insult, resulting in protection (Mattson, 2008; Stetler et al., 2014). IPC has been established in brain (Dhodda et al., 2004; Liu et al., 1992), heart (Rachmat et al., 2014), kidney (Tsutsui et al., 2013), and liver (Liu et al., 2014), and represents a prophylactic means of preventing ischemic damage. The idea of repeatedly inducing brief ischemia as a preventative treatment is far from translational, so pharmaceutical and nutraceutical means of PC are in demand. Many pharmacologic compounds including volatile anesthetics, omega-3- α -linolenic acid, 3-nitropropionic acid, sildenafil, LPS, HMGB1 protein, certain Toll-like receptor agonists and resveratrol were shown to induce ischemic tolerance (Dirnagl et al., 2003; Gidday, 2006; Morris et al., 2011). Of all these, resveratrol seems to be an attractive compound to induce PC as it is a polyphenol that is well-tolerated by humans.

Resveratrol pretreatment was first shown to mimic IPC in organotypic hippocampal cultures subjected to OGD (Raval et al., 2006). They further showed that this effect is mediated by SIRT1 activation and its inhibition by sirtinol mitigates resveratrol-induced neuroprotection. This is consistent with the previous studies that showed that acute resveratrol treatment induces SIRT1. A further study showed that resveratrol PC confers tolerance against global cerebral ischemia in adult rats by altering mitochondrial function via the SIRT1 target mitochondrial uncoupling protein 2 (UCP2) (Della-Morte et al., 2009). SIRT1 inhibitor sirtinol prevented UCP2 upregulation, which in conjunction with a study of enhanced neuroprotection in microglia from UCP2^{-/-} mice (De Bilbao et al., 2004), supports a role for SIRT1 in mitochondrial preservation during ischemia. However, it is noteworthy to mention that PC increases UCP2 levels (Liu et al., 2009), and UCP2 was shown to be neuroprotective against acute CNS injury (Haines et al., 2010; Mattiasson et al., 2003). A recent study showed that in primary rat neuronal/glia co-cultures, resveratrol PC increases mitochondrial nicotinamide phosphoribosyltransferase (Nampt) levels via PKC ϵ downstream to SIRT1 which might be responsible for the neuroprotection when these cells were subjected to OGD (Morris-Blanco et al., 2014). A follow-up *in vivo* study showed that Nrf2 is essential for resveratrol-mediated PC as resveratrol failed to induce tolerance against focal ischemia in Nrf2 knockout mice. These studies further highlight the importance of mitochondrial function in resveratrol-induced ischemic tolerance.

7. Estrogen receptor activation by resveratrol

Resveratrol is thought to mimic estrogen and is capable of activating α and β estrogen receptors (ER α and ER β) (Bowers et al., 2000; Gehm et al., 1997). This has relevance to

ischemic stroke which affects males more severely than females (Alkayed et al., 1998; Shin et al., 2010). Both estrogen and estradiol are shown to be neuroprotective after experimental ischemic stroke through the activation of ER α (Dubal et al., 2006; Elzer et al., 2010; Westberry et al., 2008). The protection afforded by estrogens in females is known to be attenuated after menopause (Cai et al., 2014).

Two recent studies explored the connection between resveratrol and ER-mediated neuroprotection after stroke. First, a rat model of permanent focal cerebral ischemia was used to show that resveratrol PC confers no additional neuroprotection when combined with a non-specific ER agonist (Saleh et al., 2010). They further showed that resveratrol PC increases the levels of stress proteins GRP94 and GRP74, heat shock protein HSP70 and m-calpain in the same model. A follow-up study showed that RPC-mediated neuroprotection could be inhibited by specific ER α or ER β antagonists in a transient focal cerebral ischemia model (Saleh et al., 2013). These experiments provide the rationale behind the hypothesis that resveratrol-mediated ER activation is neuroprotective against ischemic stroke. Further evidence of this phenomenon can be found in other relevant disease systems (Di Liberto et al., 2012; Shin et al., 2015; Yu et al., 2010).

8. Nitric oxide regulation by resveratrol

NO has a complex role in pathology and physiology (Forstermann and Munzel, 2006). Endothelial nitric oxide synthase (eNOS) is responsible for producing vasorelaxing NO in the endothelium, whereas inducible nitric oxide synthase (iNOS) promotes inflammatory NO as a result of immune cell activation (Zamora et al., 2000). As a gaseous free radical, the mere presence of NO can be an indicator of oxidative/nitrosative stress. After ischemic stroke, reperfusion-mediated increase in eNOS activity can release pathological levels of NO exacerbating oxidative stress and inflammation (Malinski et al., 1993). Also, there is evidence that persistent oxidative stress can cause eNOS uncoupling, resulting in superoxide production instead of vasoprotective NO (Li and Förstermann, 2013). Resveratrol has been shown to increase NO production in several systems (Elías et al., 2011; Klinge et al., 2008; Padín et al., 2012). However, the bulk of investigations into resveratrol-mediated NO regulation have been performed in the context of the vasculature (Simao et al., 2012c). Presumably, resveratrol works by increasing eNOS expression and activity (Wallerath et al., 2002) and by preventing eNOS uncoupling (Xia et al., 2010). In a rat model of focal ischemia, resveratrol treatment was shown to induce eNOS and concomitantly inhibit iNOS, resulting in increased production of vasoprotective NO and decreased production of neurotoxic NO (Tsai et al., 2007). Treatment of N9 microglia by resveratrol was also shown to decrease LPS-stimulated iNOS induction, leading to decreased microglial NO that potentially attenuates inflammation (Bi et al., 2005).

9. Anti-excitotoxic effects of resveratrol

Glutamate excitotoxicity is a major mechanism that kills neurons after stroke as well as TBI and SCI (Choi, 1992; Hazell, 2007; Obrenovitch and Urenjak, 1997). Resveratrol was first shown to be neuroprotective against kainate-induced excitotoxicity in rats *in vivo* (Virgili and Contestabile, 2000). Resveratrol has been shown to directly bind and inhibit excitotoxic

activation of post-synaptic kainate and NMDA receptors without altering the presynaptic volleys or postsynaptic membrane properties of rat CA1 hippocampal neurons following exposure to glutamate, kainate and NMDA (Gao et al., 2006b). Whereas, following transient focal ischemia in rats, chronic resveratrol treatment (once daily for seven days) was shown to alter the release of neurotransmitters and neuromodulators, notably glutamate and aspartate, resulting in decreased excitotoxic damage (Li et al., 2010). Tangentially, resveratrol treatment was also shown to further mitigate excitotoxicity by preventing Na⁺/K⁺ pump dysfunction in adult rats after global ischemia (Simao et al., 2011) and weight-drop induced SCI (Yang and Piao, 2003).

10. Matrix metalloproteinase regulation by resveratrol

MMPs are known to be responsible for remodeling the extracellular matrix (ECM) and are particularly important for restoring the neurovascular unit after an injury (Wang et al., 2006). However, overexpression of certain MMPs, particularly MMP-9, can exacerbate BBB disruption and secondary brain damage after ischemia, as shown by a mouse model of transient focal ischemia, in which MMP-9 knockout mice exhibited improved tight junction protein ZO-1 levels, decreased infarct size, and decreased Evans blue leakage (Asahi et al., 2001). This result is corroborated in a rat cortical impact model of TBI, in which treatment with a nonspecific MMP inhibitor resulted in decreased edema and decreased Evans blue leakage (Shigemori et al., 2006). MMP-9 is also implicated in hemorrhagic transformation after stroke (Lakhan et al., 2013; Ramos-Fernandez et al., 2011). Resveratrol treatment was shown to prevent the MMP-9 expression and activation after transient focal ischemia resulting in neuroprotection (Gao et al., 2006a). Resveratrol-induced decrease in MMP-9 after ischemia was suggested to be due to PPAR α activation (Cheng et al., 2009) and/or ERK inactivation (Gao et al., 2014). Resveratrol has also been shown to decrease MMP-9 levels in a rat perforation model of SAH, resulting in improved BBB integrity and decreased edema (Shao et al., 2014). This study suggested that NF- κ B inhibition by resveratrol was the cause of this improvement, a claim supported by studies in other systems (Ko et al., 2012; Ling et al., 2010).

11. Conclusion

The sum of the above presented investigations suggests that resveratrol is a potent pharmacological agent that can prevent secondary damage after stroke and acute CNS injury. Furthermore, resveratrol pretreatment is a viable option to induce ischemic tolerance. The beneficial effects of resveratrol are mediated synergistically by multiple major pathways that control inflammation, oxidative stress, mitochondrial function and apoptosis. The field of resveratrol-mediated neuroprotection would benefit from a combinatorial study of these major pathways in various models of acute injury. This would show which aspect of stroke/TBI/SCI/SAH pathophysiology is the most amenable to modulation. Resveratrol may also affect other aspects of stroke pathophysiology as described in section 2 (Fig. 2). Non-coding RNA and epigenetic modulation of the genome in particular are attractive candidates for future studies of this compound.

Acknowledgments

Supported partly by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number T32GM081061. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

- Agrawal M, Kumar V, Kashyap MP, Khanna VK, Randhawa GS, Pant AB. Ischemic insult induced apoptotic changes in PC12 cells: protection by trans resveratrol. *European journal of pharmacology*. 2011; 666:5–11. [PubMed: 21620820]
- Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD. Gender-linked brain injury in experimental stroke. *Stroke; a journal of cerebral circulation*. 1998; 29:159–166.
- Andrabi SA, Spina MG, Lorenz P, Ebmeyer U, Wolf G, Horn TF. Oxyresveratrol (trans-2,3',4,5'-tetrahydroxystilbene) is neuroprotective and inhibits the apoptotic cell death in transient cerebral ischemia. *Brain research*. 2004; 1017:98–107. [PubMed: 15261105]
- Asahi M, Wang X, Mori T, Sumii T, Jung JC, Moskowitz MA, Fini ME, Lo EH. Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood–brain barrier and white matter components after cerebral ischemia. *The Journal of neuroscience*. 2001; 21:7724–7732. [PubMed: 11567062]
- Ates O, Cayli S, Altinoz E, Gurses I, Yucel N, Kocak A, Yologlu S, Turkoz Y. Effects of resveratrol and methylprednisolone on biochemical, neurobehavioral and histopathological recovery after experimental spinal cord injury. *Acta pharmacologica Sinica*. 2006; 27:1317–1325.
- Ates O, Cayli S, Altinoz E, Gurses I, Yucel N, Sener M, Kocak A, Yologlu S. Neuroprotection by resveratrol against traumatic brain injury in rats. *Molecular and cellular biochemistry*. 2007; 294:137–144. [PubMed: 16924419]
- Bains M, Hall ED. Antioxidant therapies in traumatic brain and spinal cord injury. *Biochimica et biophysica acta*. 2012; 1822:675–684. [PubMed: 22080976]
- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*. 2006; 444:337–342. [PubMed: 17086191]
- Bi XL, Yang JY, Dong YX, Wang JM, Cui YH, Ikeshima T, Zhao YQ, Wu CF. Resveratrol inhibits nitric oxide and TNF- α production by lipopolysaccharide-activated microglia. *International Immunopharmacology*. 2005; 5:185–193. [PubMed: 15589480]
- Borra MT, Smith BC, Denu JM. Mechanism of human SIRT1 activation by resveratrol. *The Journal of biological chemistry*. 2005; 280:17187–17195. [PubMed: 15749705]
- Bowers JL V, Tyulmenkov V, Jernigan SC, Klinge CM. Resveratrol Acts as a Mixed Agonist/Antagonist for Estrogen Receptors α and β 1. *Endocrinology*. 2000; 141:3657–3667. [PubMed: 11014220]
- Bureau G, Longpré F, Martinoli MG. Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation. *Journal of neuroscience research*. 2008; 86:403–410. [PubMed: 17929310]
- Cai M, Ma YL, Qin P, Li Y, Zhang LX, Nie H, Peng Z, Dong H, Dong HL, Hou WG. The loss of estrogen efficacy against cerebral ischemia in aged postmenopausal female mice. *Neuroscience letters*. 2014; 558:115–119. [PubMed: 24246902]
- Calleri E, Pochetti G, Dossou KSS, Laghezza A, Montanari R, Capelli D, Prada E, Loiodice F, Massolini G, Bernier M, Moaddel R. Resveratrol and Its Metabolites Bind to PPARs. *ChemBioChem*. 2014; 15:1154–1160. [PubMed: 24796862]
- Candelario-Jalil E, de Oliveira AC, Graf S, Bhatia HS, Hull M, Munoz E, Fiebich BL. Resveratrol potently reduces prostaglandin E2 production and free radical formation in lipopolysaccharide-activated primary rat microglia. *Journal of neuroinflammation*. 2007; 4:25. [PubMed: 17927823]
- Chen CY, Jang JH, Li MH, Surh YJ. Resveratrol upregulates heme oxygenase-1 expression via activation of NF-E2-related factor 2 in PC12 cells. *Biochemical and Biophysical Research Communications*. 2005; 331:993–1000. [PubMed: 15882976]

- Cheng G, Zhang X, Gao D, Jiang X, Dong W. Resveratrol inhibits MMP-9 expression by upregulating PPAR alpha expression in an oxygen glucose deprivation-exposed neuron model. *Neuroscience letters*. 2009; 451:105–108. [PubMed: 19135132]
- Choi DW. Excitotoxic cell death. *Journal of neurobiology*. 1992; 23:1261–1276.
- Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:7217–7222. [PubMed: 17438283]
- De Bilbao F, Arsenijevic D, Vallet P, Hjelle OP, Ottersen OP, Bouras C, Raffin Y, Abou K, Langhans W, Collins S. Resistance to cerebral ischemic injury in UCP2 knockout mice: evidence for a role of UCP2 as a regulator of mitochondrial glutathione levels. *Journal of neurochemistry*. 2004; 89:1283–1292. [PubMed: 15147521]
- Delerive P, Gervois P, Fruchart JC, Staels B. Induction of IkB α expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor- α activators. *Journal of Biological Chemistry*. 2000; 275:36703–36707. [PubMed: 10980195]
- Della-Morte D, Dave KR, DeFazio RA, Bao YC, Raval AP, Perez-Pinzon MA. Resveratrol pretreatment protects rat brain from cerebral ischemic damage via a sirtuin 1-uncoupling protein 2 pathway. *Neuroscience*. 2009; 159:993–1002. [PubMed: 19356683]
- Dhodda VK, Sailor KA, Bowen KK, Vemuganti R. Putative endogenous mediators of preconditioning-induced ischemic tolerance in rat brain identified by genomic and proteomic analysis. *Journal of neurochemistry*. 2004; 89:73–89. [PubMed: 15030391]
- Di Liberto V, Mäkelä J, Korhonen L, Olivieri M, Tselykh T, Mäkiä A, Do Thi H, Belluardo N, Lindholm D, Mudò G. Involvement of estrogen receptors in the resveratrol-mediated increase in dopamine transporter in human dopaminergic neurons and in striatum of female mice. *Neuropharmacology*. 2012; 62:1011–1018.
- Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends in neurosciences*. 1999; 22:391–397. [PubMed: 10441299]
- Dirnagl U, Simon RP, Hallenbeck JM. Ischemic tolerance and endogenous neuroprotection. *Trends in neurosciences*. 2003; 26:248–254. [PubMed: 12744841]
- Domínguez C, Delgado P, Vilches A, Martín-Gallán P, Ribó M, Santamarina E, Molina C, Corbeto N, Rodríguez-Sureda V, Rosell A. Oxidative stress after thrombolysis-induced reperfusion in human stroke. *Stroke; a journal of cerebral circulation*. 2010; 41:653–660.
- Dubal DB, Rau SW, Shughrue PJ, Zhu H, Yu J, Cashion AB, Suzuki S, Gerhold LM, Bottner MB, Dubal SB. Differential modulation of estrogen receptors (ERs) in ischemic brain injury: a role for ER α in estradiol-mediated protection against delayed cell death. *Endocrinology*. 2006; 147:3076–3084.
- Elías J, Cufiñas A, García-Morales V, Orallo F, Campos-Toimil M. Trans-resveratrol simultaneously increases cytoplasmic Ca²⁺ levels and nitric oxide release in human endothelial cells. *Molecular nutrition & food research*. 2011; 55:1237–1248. [PubMed: 21710562]
- Elzer JG, Muhammad S, Wintermantel TM, Regnier-Vigouroux A, Ludwig J, Schütz G, Schwaninger M. Neuronal estrogen receptor- α mediates neuroprotection by 17 β -estradiol. *Journal of Cerebral Blood Flow & Metabolism*. 2010; 30:935–942. [PubMed: 20010956]
- Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation*. 2006; 113:1708–1714. [PubMed: 16585403]
- Gao D, Huang T, Jiang X, Hu S, Zhang L, Fei Z. Resveratrol protects primary cortical neuron cultures from transient oxygen-glucose deprivation by inhibiting MMP-9. *Molecular medicine reports*. 2014; 9:2197–2204. [PubMed: 24682241]
- Gao D, Zhang X, Jiang X, Peng Y, Huang W, Cheng G, Song L. Resveratrol reduces the elevated level of MMP-9 induced by cerebral ischemia-reperfusion in mice. *Life sciences*. 2006a; 78:2564–2570. [PubMed: 16321402]
- Gao ZB, Chen XQ, Hu GY. Inhibition of excitatory synaptic transmission by trans-resveratrol in rat hippocampus. *Brain research*. 2006b; 1111:41–47. [PubMed: 16876771]
- Gatson JW, Liu MM, Abdelfattah K, Wigginton JG, Smith S, Wolf S, Minei JP. Resveratrol decreases inflammation in the brain of mice with mild traumatic brain injury. *The journal of trauma and acute care surgery*. 2013; 74:470–474. discussion 474–475. [PubMed: 23354240]

- Gehm BD, McAndrews JM, Chien PY, Jameson JL. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. *Proceedings of the National Academy of Sciences*. 1997; 94:14138–14143.
- Gidday JM. Cerebral preconditioning and ischaemic tolerance. *Nature reviews Neuroscience*. 2006; 7:437–448. [PubMed: 16715053]
- Girbovan C, Morin L, Plamondon H. Repeated resveratrol administration confers lasting protection against neuronal damage but induces dose-related alterations of behavioral impairments after global ischemia. *Behavioural pharmacology*. 2012; 23:1–13. [PubMed: 22146698]
- Girbovan C, Plamondon H. Resveratrol downregulates type-1 glutamate transporter expression and microglia activation in the hippocampus following cerebral ischemia reperfusion in rats. *Brain research*. 2015; 1608:203–214. [PubMed: 25727173]
- Gong, Q-h; Wang, Q.; Shi, J-s; Huang, X-n; Liu, Q.; Ma, H. Inhibition of caspases and intracellular free Ca²⁺ concentrations are involved in resveratrol protection against apoptosis in rat primary neuron cultures. *Acta pharmacologica Sinica*. 2007; 28:1724–1730. [PubMed: 17959022]
- Haines BA, Mehta SL, Pratt SM, Warden CH, Li PA. Deletion of mitochondrial uncoupling protein-2 increases ischemic brain damage after transient focal ischemia by altering gene expression patterns and enhancing inflammatory cytokines. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2010; 30:1825–1833.
- Hall ED, Vaishnav RA, Mustafa AG. Antioxidant therapies for traumatic brain injury. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*. 2010; 7:51–61. [PubMed: 20129497]
- Hazell AS. Excitotoxic mechanisms in stroke: an update of concepts and treatment strategies. *Neurochemistry international*. 2007; 50:941–953. [PubMed: 17576023]
- Heynekamp JJ, Weber WM, Hunsaker LA, Gonzales AM, Orlando RA, Deck LM, Vander Jagt DL. Substituted trans-stilbenes, including analogues of the natural product resveratrol, inhibit the human tumor necrosis factor alpha-induced activation of transcription factor nuclear factor kappaB. *Journal of medicinal chemistry*. 2006; 49:7182–7189. [PubMed: 17125270]
- Hochrainer K, Jackman K, Anrather J, Iadecola C. Reperfusion rather than ischemia drives the formation of ubiquitin aggregates after middle cerebral artery occlusion. *Stroke; a journal of cerebral circulation*. 2012; 43:2229–2235.
- Huang SS, Tsai MC, Chih CL, Hung LM, Tsai SK. Resveratrol reduction of infarct size in Long-Evans rats subjected to focal cerebral ischemia. *Life sciences*. 2001; 69:1057–1065. [PubMed: 11508648]
- Inoue H. Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator-activated receptor α in mice. *Neuroscience letters*. 2003
- Jäger S, Handschin C, Pierre JS- Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 α . *Proceedings of the National Academy of Sciences*. 2007; 104:12017–12022.
- Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*. 1997; 275:218–220. [PubMed: 8985016]
- Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: Mechanisms of activation and dysregulation in cancer. *Redox biology*. 2013; 1:45–49.
- Kaplan S, Bisleri G, Morgan JA, Cheema FH, Oz MC. Resveratrol, a natural red wine polyphenol, reduces ischemia-reperfusion-induced spinal cord injury. *The Annals of thoracic surgery*. 2005; 80:2242–2249. [PubMed: 16305881]
- Karalis F, Soubasi V, Georgiou T, Nakas CT, Simeonidou C, Guiba-Tziampiri O, Spandou E. Resveratrol ameliorates hypoxia/ischemia-induced behavioral deficits and brain injury in the neonatal rat brain. *Brain research*. 2011; 1425:98–110. [PubMed: 22018692]
- Karaoglan A, Akdemir O, Barut S, Kokturk S, Uzun H, Tasyurekli M, Colak A. The effects of resveratrol on vasospasm after experimental subarachnoidal hemorrhage in rats. *Surgical neurology*. 2008; 70:337–343.
- Keshewani V, Atif F, Yousuf S, Agrawal SK. Resveratrol protects spinal cord dorsal column from hypoxic injury by activating Nrf-2. *Neuroscience*. 2013; 241:80–88. [PubMed: 23523995]

- Khodr B, Khalil Z. Modulation of inflammation by reactive oxygen species: implications for aging and tissue repair. *Free Radical Biology and Medicine*. 2001; 30:1–8. [PubMed: 11134890]
- Klinge CM, Wickramasinghe NS, Ivanova MM, Dougherty SM. Resveratrol stimulates nitric oxide production by increasing estrogen receptor alpha- Src -caveolin-1 interaction and phosphorylation in human umbilical vein endothelial cells. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2008; 22:2185–2197. [PubMed: 18296501]
- Ko HS, Lee HJ, Kim SH, Lee EO. Piceatannol suppresses breast cancer cell invasion through the inhibition of MMP-9: involvement of PI3K/AKT and NF- κ B pathways. *Journal of agricultural and food chemistry*. 2012; 60:4083–4089.
- Kocina P. Body composition of spinal cord injured adults. *Sports Medicine*. 1997; 23:48–60.
- Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell*. 2006; 127:1109–1122. [PubMed: 17112576]
- Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. *Journal of translational medicine*. 2009; 7:97.
- Lakhan SE, Kirchgessner A, Tepper D, Leonard A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Frontiers in neurology*. 2013; 4
- Lanzillotta A, Pignataro G, Branca C, Cuomo O, Sarnico I, Benarese M, Annunziato L, Spano P, Pizzi M. Targeted acetylation of NF- κ B/RelA and histones by epigenetic drugs reduces post-ischemic brain injury in mice with an extended therapeutic window. *Neurobiology of disease*. 2013; 49:177–189.
- Lee, Y-j; Mou, Y.; Klimanis, D.; Bernstock, JD.; Hallenbeck, JM. Global SUMOylation is a molecular mechanism underlying hypothermia-induced ischemic tolerance. *Frontiers in cellular neuroscience*. 2014; 8
- Li C, Yan Z, Yang J, Chen H, Li H, Jiang Y, Zhang Z. Neuroprotective effects of resveratrol on ischemic injury mediated by modulating the release of neurotransmitter and neuromodulator in rats. *Neurochemistry international*. 2010; 56:495–500. [PubMed: 20026214]
- Li H, Förstermann U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. *Current opinion in pharmacology*. 2013; 13:161–167. [PubMed: 23395155]
- Li Z, Pang L, Fang F, Zhang G, Zhang J, Xie M, Wang L. Resveratrol attenuates brain damage in a rat model of focal cerebral ischemia via up-regulation of hippocampal Bcl-2. *Brain research*. 2012; 1450:116–124. [PubMed: 22410291]
- Lin CJ, Chen TH, Yang LY, Shih CM. Resveratrol protects astrocytes against traumatic brain injury through inhibiting apoptotic and autophagic cell death. *Cell death & disease*. 2014; 5:e1147. [PubMed: 24675465]
- Lin HY, Tang HY, Davis FB, Davis PJ. Resveratrol and apoptosis. *Annals of the New York Academy of Sciences*. 2011; 1215:79–88. [PubMed: 21261644]
- Ling H, Yang H, Tan SH, Chui WK, Chew EH. 6-Shogaol, an active constituent of ginger, inhibits breast cancer cell invasion by reducing matrix metalloproteinase-9 expression via blockade of nuclear factor- κ B activation. *British journal of pharmacology*. 2010; 161:1763–1777. [PubMed: 20718733]
- Liu A, Fang H, Wei W, Dirsch O, Dahmen U. Ischemic Preconditioning Protects Against Liver Ischemia/Reperfusion Injury via Heme Oxygenase-1-Mediated Autophagy. *Critical care medicine*. 2014; 42:e762–e771. [PubMed: 25402296]
- Liu C, Shi Z, Fan L, Zhang C, Wang K, Wang B. Resveratrol improves neuron protection and functional recovery in rat model of spinal cord injury. *Brain research*. 2011; 1374:100–109. [PubMed: 21111721]
- Liu Y, Chen L, Xu X, Vicaut E, Sercombe R. Both ischemic preconditioning and ghrelin administration protect hippocampus from ischemia/reperfusion and upregulate uncoupling protein-2. *BMC physiology*. 2009; 9:17. [PubMed: 19772611]
- Liu Y, Kato H, Nakata N, Kogure K. Protection of rat hippocampus against ischemic neuronal damage by pretreatment with sublethal ischemia. *Brain research*. 1992; 586:121–124. [PubMed: 1380876]
- Lorenz P, Roychowdhury S, Engelmann M, Wolf G, Horn TF. Oxyresveratrol and resveratrol are potent antioxidants and free radical scavengers: effect on nitrosative and oxidative stress derived

- from microglial cells. Nitric oxide : biology and chemistry/official journal of the Nitric Oxide Society. 2003; 9:64–76. [PubMed: 14623172]
- Lu Z, Xu X, Hu X, Fassett J, Zhu G, Tao Y, Li J, Huang Y, Zhang P, Zhao B. PGC-1 α regulates expression of myocardial mitochondrial antioxidants and myocardial oxidative stress after chronic systolic overload. *Antioxidants & redox signaling*. 2010; 13:1011–1022. [PubMed: 20406135]
- Malinski T, Bailey F, Zhang Z, Chopp M. Nitric oxide measured by a porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. *Journal of Cerebral Blood Flow & Metabolism*. 1993; 13:355–358. [PubMed: 8478395]
- Manzanero S, Santro T, Arumugam TV. Neuronal oxidative stress in acute ischemic stroke: sources and contribution to cell injury. *Neurochemistry international*. 2013; 62:712–718. [PubMed: 23201332]
- Masel BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. *Journal of neurotrauma*. 2010; 27:1529–1540. [PubMed: 20504161]
- Mattiasson G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S, Warden CH, Castilho RF, Melcher T, Gonzalez-Zulueta M. Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. *Nature medicine*. 2003; 9:1062–1068.
- Mattson MP. Hormesis defined. *Ageing research reviews*. 2008; 7:1–7. [PubMed: 18162444]
- Mohamed HE, El-Swefy SE, Hasan RA, Hasan AA. Neuroprotective effect of resveratrol in diabetic cerebral ischemic-reperfused rats through regulation of inflammatory and apoptotic events. *Diabetology and Metabolic Syndrome*. 2014; 6:88–101. [PubMed: 25191525]
- Morris-Blanco KC, Cohan CH, Neumann JT, Sick TJ, Perez-Pinzon MA. Protein kinase C epsilon regulates mitochondrial pools of Namp1 and NAD following resveratrol and ischemic preconditioning in the rat cortex. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2014; 34:1024–1032.
- Morris KC, Lin HW, Thompson JW, Perez-Pinzon MA. Pathways for ischemic cytoprotection: role of sirtuins in caloric restriction, resveratrol, and ischemic preconditioning. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2011; 31:1003–1019.
- Nakka VP, Lang BT, Lenschow DJ, Zhang DE, Dempsey RJ, Vemuganti R. Increased cerebral protein ISGylation after focal ischemia is neuroprotective. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2011; 31:2375–2384.
- Nakka VP, Prakash-babu P, Vemuganti R. Crosstalk between endoplasmic reticulum stress, oxidative stress, and autophagy: potential therapeutic targets for acute CNS injuries. *Molecular neurobiology*. 2014:1–13.
- Nolan S. Traumatic brain injury: a review. *Critical Care Nursing Quarterly*. 2005; 28:188–194. [PubMed: 15875448]
- Obrenovitch TP, Urenjak J. Altered glutamatergic transmission in neurological disorders: from high extracellular glutamate to excessive synaptic efficacy. *Progress in neurobiology*. 1997; 51:39–87. [PubMed: 9044428]
- Padín JF, de Diego AM, Fernández-Morales JC, Merino C, Maroto M, Calvo-Gallardo E, Arranz JA, Yáñez M, García AG. Resveratrol augments nitric oxide generation and causes store calcium release in chromaffin cells. *European journal of pharmacology*. 2012; 685:99–107. [PubMed: 22498000]
- Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H, Ke H, Rehmann H, Taussig R, Brown AL, Kim MK, Beaven MA, Burgin AB, Manganiello V, Chung JH. Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. *Cell*. 2012; 148:421–433. [PubMed: 22304913]
- Quincozes-Santos A, Bobermin LD, Latini A, Wajner M, Souza DO, Goncalves CA, Gottfried C. Resveratrol protects C6 astrocyte cell line against hydrogen peroxide-induced oxidative stress through heme oxygenase 1. *PloS one*. 2013; 8:e64372. [PubMed: 23691207]
- Rachmat J, Sastroasmoro S, Suyatna FD, Soejono G. Ischemic preconditioning reduces apoptosis in open heart surgery. *Asian Cardiovascular and Thoracic Annals*. 2014; 22:276–283. [PubMed: 24585902]

- Ramos-Fernandez M, Bellolio MF, Stead LG. Matrix metalloproteinase-9 as a marker for acute ischemic stroke: a systematic review. *Journal of Stroke and Cerebrovascular Diseases*. 2011; 20:47–54. [PubMed: 21044610]
- Raval AP, Dave KR, Perez-Pinzon MA. Resveratrol mimics ischemic preconditioning in the brain. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2006; 26:1141–1147.
- Rege SD, Kumar S, Wilson DN, Tamura L, Geetha T, Mathews ST, Huggins KW, Broderick TL, Babu JR. Resveratrol protects the brain of obese mice from oxidative damage. *Oxidative medicine and cellular longevity*. 2013; 2013:419092.
- Ren J, Fan C, Chen N, Huang J, Yang Q. Resveratrol pretreatment attenuates cerebral ischemic injury by upregulating expression of transcription factor Nrf2 and HO-1 in rats. *Neurochemical research*. 2011; 36:2352–2362. [PubMed: 21850487]
- Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1 α and SIRT1. *Nature*. 2005; 434:113–118. [PubMed: 15744310]
- Sakata Y, Zhuang H, Kwansa H, Koehler RC, Dore S. Resveratrol protects against experimental stroke: putative neuroprotective role of heme oxygenase 1. *Experimental neurology*. 2010; 224:325–329. [PubMed: 20381489]
- Saleh MC, Connell BJ, Saleh TM. Resveratrol preconditioning induces cellular stress proteins and is mediated via NMDA and estrogen receptors. *Neuroscience*. 2010; 166:445–454. [PubMed: 20040366]
- Saleh MC, Connell BJ, Saleh TM. Resveratrol induced neuroprotection is mediated via both estrogen receptor subtypes, ER(α) and ER(β). *Neuroscience letters*. 2013; 548:217–221. [PubMed: 23748073]
- Shang YJ, Qian YP, Liu XD, Dai F, Shang XL, Jia WQ, Liu Q, Fang JG, Zhou B. Radical-scavenging activity and mechanism of resveratrol-oriented analogues: influence of the solvent, radical, and substitution. *The Journal of organic chemistry*. 2009; 74:5025–5031. [PubMed: 19472994]
- Shao AW, Wu HJ, Chen S, Ammar AB, Zhang JM, Hong Y. Resveratrol attenuates early brain injury after subarachnoid hemorrhage through inhibition of NF-kappaB-dependent inflammatory/MMP-9 pathway. *CNS neuroscience & therapeutics*. 2014; 20:182–185. [PubMed: 24279692]
- Shigemori, Y.; Katayama, Y.; Mori, T.; Maeda, T.; Kawamata, T. *Brain Edema*. Vol. XIII. Springer; 2006. Matrix metalloproteinase-9 is associated with blood-brain barrier opening and brain edema formation after cortical contusion in rats; p. 130-133.
- Shin JA, Lee H, Lim YK, Koh Y, Choi JH, Park EM. Therapeutic effects of resveratrol during acute periods following experimental ischemic stroke. *Journal of neuroimmunology*. 2010; 227:93–100. [PubMed: 20655115]
- Shin JA, Lee KE, Kim HS, Park EM. Acute resveratrol treatment modulates multiple signaling pathways in the ischemic brain. *Neurochemical research*. 2012; 37:2686–2696. [PubMed: 22878646]
- Shin JA, Oh S, Ahn JH, Park EM. Estrogen receptor-mediated resveratrol actions on blood-brain barrier of ovariectomized mice. *Neurobiology of aging*. 2015; 36:993–1006. [PubMed: 25448605]
- Siemann E, Creasy L. Concentration of the phytoalexin resveratrol in wine. *American Journal of Enology and Viticulture*. 1992; 43:49–52.
- Silva NA, Sousa N, Reis RL, Salgado AJ. From basics to clinical: a comprehensive review on spinal cord injury. *Progress in neurobiology*. 2014; 114:25–57. [PubMed: 24269804]
- Simao F, Matte A, Matte C, Soares FM, Wyse AT, Netto CA, Salbego CG. Resveratrol prevents oxidative stress and inhibition of Na(+)-K(+)-ATPase activity induced by transient global cerebral ischemia in rats. *The Journal of nutritional biochemistry*. 2011; 22:921–928. [PubMed: 21208792]
- Simao F, Matte A, Pagnussat AS, Netto CA, Salbego CG. Resveratrol preconditioning modulates inflammatory response in the rat hippocampus following global cerebral ischemia. *Neurochemistry international*. 2012a; 61:659–665. [PubMed: 22709670]

- Simao F, Matte A, Pagnussat AS, Netto CA, Salbego CG. Resveratrol prevents CA1 neurons against ischemic injury by parallel modulation of both GSK-3 β and CREB through PI3- K/Akt pathways. *The European journal of neuroscience*. 2012b; 36:2899–2905. [PubMed: 22817531]
- Simao F, Pagnussat AS, Seo JH, Navaratna D, Leung W, Lok J, Guo S, Waeber C, Salbego CG, Lo EH. Pro-angiogenic effects of resveratrol in brain endothelial cells: nitric oxide-mediated regulation of vascular endothelial growth factor and metalloproteinases. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2012c; 32:884–895.
- Singleton RH, Yan HQ, Fellows-Mayle W, Dixon CE. Resveratrol attenuates behavioral impairments and reduces cortical and hippocampal loss in a rat controlled cortical impact model of traumatic brain injury. *Journal of neurotrauma*. 2010; 27:1091–1099. [PubMed: 20560755]
- Sinha K, Chaudhary G, Kumar Gupta Y. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life sciences*. 2002; 71:655–665. [PubMed: 12072154]
- Song J, Cheon SY, Jung W, Lee WT, Lee JE. Resveratrol induces the expression of interleukin-10 and brain-derived neurotrophic factor in BV2 microglia under hypoxia. *International journal of molecular sciences*. 2014; 15:15512–15529.
- Sonmez U, Sonmez A, Erbil G, Tekmen I, Baykara B. Neuroprotective effects of resveratrol against traumatic brain injury in immature rats. *Neuroscience letters*. 2007; 420:133–137. [PubMed: 17531385]
- Spasi MR, Callaerts P, Norga KK. AMP-activated protein kinase (AMPK) molecular crossroad for metabolic control and survival of neurons. *The Neuroscientist*. 2009; 15:309–316. [PubMed: 19359670]
- St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, Spiegelman BM. Suppression of Reactive Oxygen Species and Neurodegeneration by the PGC-1 Transcriptional Coactivators. *Cell*. 2006; 127:397–408. [PubMed: 17055439]
- St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, Spiegelman BM. Bioenergetic analysis of peroxisome proliferator-activated receptor γ coactivators 1 α and 1 β (PGC-1 α and PGC-1 β) in muscle cells. *Journal of Biological Chemistry*. 2003; 278:26597–26603. [PubMed: 12734177]
- Stetler RA, Leak RK, Gan Y, Li P, Zhang F, Hu X, Jing Z, Chen J, Zigmond MJ, Gao Y. Preconditioning provides neuroprotection in models of CNS disease: paradigms and clinical significance. *Progress in neurobiology*. 2014; 114:58–83. [PubMed: 24389580]
- Takaoka M. Of the phenolic substances of white hellebore (*Veratrum grandiflorum* Loes. fil.). *J Faculty Sci Hokkaido Imperial University*. 1940; 3:1–16.
- Tang BL. Resveratrol is neuroprotective because it is not a direct activator of Sirt1-A hypothesis. *Brain research bulletin*. 2010; 81:359–361. [PubMed: 20026255]
- Tsai SK, Hung LM, Fu YT, Cheng H, Nien MW, Liu HY, Zhang FB, Huang SS. Resveratrol neuroprotective effects during focal cerebral ischemia injury via nitric oxide mechanism in rats. *Journal of vascular surgery*. 2007; 46:346–353. [PubMed: 17600658]
- Tsai YF, Liu FC, Lau YT, Yu HP. Role of Akt-dependent pathway in resveratrol-mediated cardioprotection after trauma-hemorrhage. *The Journal of surgical research*. 2012; 176:171–177. [PubMed: 21764074]
- Tsutsui H, Tanaka R, Yamagata M, Yukimura T, Ohkita M, Matsumura Y. Protective effect of ischemic preconditioning on ischemia/reperfusion-induced acute kidney injury through sympathetic nervous system in rats. *European journal of pharmacology*. 2013; 718:206–212. [PubMed: 24036256]
- Ungvari Z, Bagi Z, Feher A, Recchia FA, Sonntag WE, Pearson K, de Cabo R, Csiszar A. Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. 2010:H18–H24.
- Virgili M, Contestabile A. Partial neuroprotection of in vivo excitotoxic brain damage by chronic administration of the red wine antioxidant agent, trans-resveratrol in rats. *Neuroscience letters*. 2000; 281:123–126. [PubMed: 10704758]

- Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, Förstermann U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation*. 2002; 106:1652–1658. [PubMed: 12270858]
- Wang HD, Shi YM, Li L, Guo JD, Zhang YP, Hou SX. Treatment with resveratrol attenuates sublesional bone loss in spinal cord-injured rats. *British journal of pharmacology*. 2013; 170:796–806. [PubMed: 23848300]
- Wang L, Zhang ZG, Zhang RL, Gregg SR, Hozeska-Solgot A, Le Tourneau Y, Wang Y, Chopp M. Matrix metalloproteinase 2 (MMP2) and MMP9 secreted by erythropoietin-activated endothelial cells promote neural progenitor cell migration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006; 26:5996–6003. [PubMed: 16738242]
- Wang M, Huang H, Hsieh S, Jeng K, Kuo J. Resveratrol inhibits interleukin-6 production in cortical mixed glial cells under hypoxia/hypoglycemia followed by reoxygenation. *Journal of neuroimmunology*. 2001; 112:28–34. [PubMed: 11108930]
- Wang T, Gu J, Wu PF, Wang F, Xiong Z, Yang YJ, Wu WN, Dong LD, Chen JG. Protection by tetrahydroxystilbene glucoside against cerebral ischemia: involvement of JNK, SIRT1, and NF-kappaB pathways and inhibition of intracellular ROS/RNS generation. *Free radical biology & medicine*. 2009; 47:229–240. [PubMed: 19272442]
- West T, Atzeva M, Holtzman DM. Pomegranate polyphenols and resveratrol protect the neonatal brain against hypoxic-ischemic injury. *Developmental neuroscience*. 2007; 29:363–372. [PubMed: 17762204]
- Westberry JM, Prewitt AK, Wilson ME. Epigenetic regulation of the estrogen receptor alpha promoter in the cerebral cortex following ischemia in male and female rats. *Neuroscience*. 2008; 152:982–989. [PubMed: 18353557]
- Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature*. 2004; 430:686–689. [PubMed: 15254550]
- Xia N, Daiber A, Habermeyer A, Closs EI, Thum T, Spanier G, Lu Q, Oelze M, Torzewski M, Lackner KJ, Munzel T, Forstermann U, Li H. Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. *The Journal of pharmacology and experimental therapeutics*. 2010; 335:149–154. [PubMed: 20610621]
- Yang YB, Piao YJ. Effects of resveratrol on secondary damages after acute spinal cord injury in rats. *Acta pharmacologica Sinica*. 2003; 24:703–710. [PubMed: 12852839]
- Yang Y, Duan W, Li Y, Yan J, Yi W, Liang Z, Wang N, Yi D, Jin Z. New role of silent information regulator 1 in cerebral ischemia. *Neurobiology of aging*. 2013; 34:2879–2888. [PubMed: 23855981]
- Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. Modulation of NF-kB-dependent transcription and cell survival by the SIRT1 deacetylase. *The EMBO journal*. 2004; 23:2369–2380. [PubMed: 15152190]
- Yousuf S, Atif F, Ahmad M, Hoda N, Ishrat T, Khan B, Islam F. Resveratrol exerts its neuroprotective effect by modulating mitochondrial dysfunctions and associated cell death during cerebral ischemia. *Brain research*. 2009; 1250:242–253. [PubMed: 19027723]
- Yu HP, Hwang TL, Yen CH, Lau YT. Resveratrol prevents endothelial dysfunction and aortic superoxide production after trauma hemorrhage through estrogen receptor-dependent hemoxygenase-1 pathway. *Critical care medicine*. 2010; 38:1147–1154. [PubMed: 20081535]
- Zamin LL, Dillenburg-Pilla P, Argenta-Comiran R, Horn AP, Simao F, Nassif M, Gerhardt D, Frozza RL, Salbego C. Protective effect of resveratrol against oxygen-glucose deprivation in organotypic hippocampal slice cultures: Involvement of PI3-K pathway. *Neurobiology of disease*. 2006; 24:170–182. [PubMed: 16860989]
- Zamora R, Vodovotz Y, Billiar TR. Inducible nitric oxide synthase and inflammatory diseases. *Molecular Medicine*. 2000; 6:347. [PubMed: 10952018]
- Zhang M, An C, Gao Y, Leak RK, Chen J, Zhang F. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. *Progress in neurobiology*. 2013; 100:30–47. [PubMed: 23025925]

- Zhou XM, Zhou ML, Zhang XS, Zhuang Z, Li T, Shi JX, Zhang X. Resveratrol prevents neuronal apoptosis in an early brain injury model. *The Journal of surgical research*. 2014; 189:159–165. [PubMed: 24602480]
- Zhuang H, Kim Y-S, Koehler RC, DorÉ S. Potential Mechanism by Which Resveratrol, a Red Wine Constituent, Protects Neurons. *Annals of the New York Academy of Sciences*. 2003; 993:276–286. [PubMed: 12853318]
- Zweier JL, Talukder MH. The role of oxidants and free radicals in reperfusion injury. *Cardiovascular research*. 2006; 70:181–190. [PubMed: 16580655]

Fig. 1.

Resveratrol induces a neuroprotective state via several disparate pathways. The exact mechanism of resveratrol-mediated neuroprotection is not yet understood, but the downstream anti-oxidative, anti-inflammatory and anti-apoptotic effectors have been well documented. This diagram illustrates the factors responsible for inducing a pro-survival state after resveratrol treatment in the CNS. Note that some effectors, particularly SIRT1 and AMPK can be activated or inhibited by more than one pathway. Arrows with a point indicate activation, while arrows with a flat tip indicate inhibition. White arrows indicate activation/inhibition via an indirect or poorly understood mechanism. Green = role in inflammation. Pink = role in oxidative stress. Blue = role in apoptosis. White = transcription factor or pathway intermediary.

Figure 2.

Many pathophysiologic mechanisms that start within minutes and continue for days synergistically promote neuronal death following ischemic and traumatic injuries to CNS. Resveratrol treatment was shown to prevent secondary brain damage by curtailing several of these mechanisms, including excitotoxicity, edema, apoptosis, oxidative and nitrosative stress, inflammation and BBB disruption (shown by a purple box). Effects of resveratrol on mechanisms such as non-coding RNAs and epigenetics that are also important modulators of secondary brain damage were not yet evaluated.