Resveratrol and the eye: activity and molecular mechanisms

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Abstract

Purpose
Alcohol consumption is inversely correlated with the incidence of cardiovascular disease. It is thought that red wine is specifically responsible for these cardiovascular benefits, due to its ability to reduce vascular inflammation, facilitate vasorelaxation and inhibit angiogenesis. This is because of its high polyphenolic content. Resveratrol is the main biologically active polyphenol within red wine. Owing to its vascular enhancing properties, resveratrol may be effective in the microcirculation of the eye, thereby helping prevent ocular diseases such as age-related macular degeneration, diabetic retinopathy and glaucoma. Such conditions are accountable for worldwide prevalence of visual loss.

Method
A review of the relevant literature was conducted on the ScienceDirect, Web of Science and PubMed databases. Key words used to carry out the searches included ‘red wine’, ‘polyphenols’, ‘resveratrol’, ‘eye’ and ‘ocular’. Articles relating to the effects of resveratrol on the eye were reviewed.

Results
The protective effects of resveratrol within the eye are extensive. It has been demonstrated to have anti-oxidant, anti-apoptotic, anti-tumourogenic, anti-inflammatory, anti-angiogenic and vasorelaxant properties. There are potential benefits of resveratrol supplementation across a wide range of ocular diseases. The molecular mechanisms underlying these protective actions are diverse.

Conclusion
Evidence suggests that resveratrol may have potential in the treatment of several ocular diseases. However while there are many studies indicating plausible biological mechanisms using animal models and in vitro retinal cells there is a paucity of human research. The evidence base for the use of resveratrol in the management of ocular diseases needs to be increased before recommendations can be made for the use of resveratrol as an ocular supplement.

Keywords: age-related macular degeneration, alcohol, diabetic retinopathy, polyphenols, red wine, resveratrol, retinopathy of prematurity

Introduction

There has been considerable interest in alcohol and the dangers it poses to good health. Less attention has been directed towards the potential benefits of alcohol consumption. The first documentation presenting the favourable effects of alcohol dates back to 1786, when Heberden discovered its ability to palliate angina pectoris [1]. Subsequently, a plethora of research has been performed to elucidate the potential cardiovascular benefits of alcohol.

It appears that this paradoxical view of alcohol consumption is down to the *amount* of alcohol consumed. This can be explained by the J-shaped curve shown in studies investigating the association of alcohol dose and cardiovascular health [2]. The curve depicts possible cardiovascular benefits at a light to moderate alcohol intake, in contrast to potentially hazardous effects with heavy consumption.

In 1979, research established that red wine, specifically, was responsible for the inverse correlation between alcohol consumption and cardiovascular disease [3]. This finding was further supported by the 'French Paradox', a theory conceptualised by Renaud *et al* [4]. This theory attempts to explain the comparatively low incidence of cardiovascular disease among the French population, despite their considerably high dietary intake of saturated fat. It was thought that red wine had greater beneficial potential for health than other alcoholic beverages due to its high content of polyphenolic compounds [5]. These compounds possess antioxidant properties, which account for their protective effect. The majority of the grape-derived polyphenols reside in the skin and seeds of the berry, both of which are removed during the production of white wine. Hence, red wine has comparatively higher polyphenolic content and subsequently greater anti-oxidant properties [6].

Current research has been focussed on the systemic effects of red wine, much of which has revolved around cardiovascular benefits [7]. Red wine has also, however, been associated with a decreased incidence of certain types of cancers including breast cancer [8] and lung cancer [9]. There have been fewer studies investigating the relationship between red wine and the eye.

Interest has arisen due to its cardioprotective action upon vasculature i.e. ability to inhibit angiogenesis, prevent inflammation and facilitate vaso-relaxation [7], all of which lead to increased blood perfusion of a biological tissue. Impaired blood flow and subsequent ischaemic changes are key pathological features of several ocular diseases including age-related macular degeneration (AMD), diabetic retinopathy (DR) and glaucoma. It is evident therefore, that red wine has the potential to prevent the onset and progression of such diseases.

The last decade has seen a growing interest in the effects of resveratrol on the eye, particularly in terms of disease prevention. This naturally occurring polyphenol is thought to be the principal biologically active substance within red wine, due to its comparatively higher concentration in red grapes as opposed to white [10]. In addition, resveratrol has been reported to delay the progression of several age-related diseases, such as Alzheimer's disease [11]. The potential beneficial effects of this substance within the body are diverse.

This literature review aims to give an overview of the mechanisms by which resveratrol may prevent the progression and/or onset of ocular diseases that are common cause of sight loss. The clinical significance of these findings will be discussed in terms of current and future direction.

Method

Relevant articles investigating the impact of resveratrol on the eye were identified. All articles considered, were obtained from peer-reviewed journals. Initially, ScienceDirect, Web of Science and PubMed databases were searched for pertinent articles between 1970 and 2013. The following key words were used to perform the search: 'alcohol', 'red wine', 'red wine polyphenols', 'polyphenols', 'resveratrol', 'trans-resveratrol'. Each of these terms were then used in combination with words including 'eye', 'ocular', 'retina' to refine the search. The abstracts of those articles considered of relevance were read and full-text copies of the articles were accessed. In addition, any papers identified as relevant from cited references within these

articles were searched using a similar procedure. Having reviewed the literature selected, information regarding the effects of resveratrol on the eye was integrated into the document.

Results

Red wine polyphenols

Red wine contains a variety of polyphenolic compounds in abundance. Most of these are extracted during fermentation of the grapes' seeds, stems and skin which have 65%, 22% and 12% polyphenolic content, respectively [12]. Figure 1 depicts the basic structural component of all polyphenolic compounds: the phenol group. The hydroxyl group present within the structure can be oxidised by proton donation to free radicals, accounting for the anti-oxidant effect of polyphenols [13].

Figure 1 about here

There are two forms of polyphenols found in red wine, namely flavonoids and non-flavonoids (table 1) [13]. The flavonoids comprise the majority of polyphenol content in red wine [14]. However, more recent research has focussed on the biological effects of the non-flavonoid resveratrol.

Table 1 about here

Resveratrol

Resveratrol (3, 4', 5-trihydroxystilbene) is a natural polyphenolic phytoalexin that is mainly found in grapes, leading to its high concentration in wines [15]. Additional sources include peanuts [15], blueberries [16], bilberries [16] and cranberries [17]. Resveratrol has a stilbene structure, meaning that the compound consists of two aromatic rings connected by a methylene bridge [18]. There exist two structurally distinct forms of resveratrol, namely cis- and trans-resveratrol (figure 2) [18]. The cis- isomeric form had not been located in grapes [18], however one study reported high amounts of the cis-isomer in wine [19]. It had originally been postulated that cis-

resveratrol was formed from its trans- isomer by ultraviolet (UV) irradiation [10], but work by Jeandet *et al.* proved that similarly high amounts of the cis-isomer were located in wine produced in the dark that had vinted in the presence of light [19].

In 1963 resveratrol was detected as the active ingredient in *Polygonum cuspidatum* roots, otherwise known as 'Ko-jo-kon' in Japanese [20]. The production of trans-resveratrol in grapevines (*Vitis vinifera*) however, was first discovered in 1976; its synthesis initiated as a method of defence against fungal attack or exposure to UV light [18]. As a result, it was generally thought that trans-resveratrol was the more biologically active isomer. Conversely, it was identified that cis-resveratrol had comparable ability to the trans- isomer in lowering lipid serum levels, therefore establishing a possible involvement for both forms of the compound in a protective role [21]. Resveratrol was found to reside only in the skin of grapes, upon synthesis in response to UV light [22]. Since pioneering reports in 1992 confirming the presence of the named phytoalexin in red wine [10], resveratrol has become a topic of considerable interest.

Figure 2 about here

Resveratrol concentration in wines

There are many factors influencing the resveratrol concentration of red wine, some of which include climate, type of grape, conditions of growth and production [10, 23, 24]. A higher content of resveratrol is found in red grapes, hence the comparatively higher concentration found in red wine as opposed to white wines [10]. The variety of grape can be further classified in terms of species, originating from different countries. Lamikanra *et al.* compared resveratrol content of red wines produced using *Vitis vinifera, Vitis labruscana and Vitis rotundifolia* (muscadine) grape cultivars, and found that wine formed from the latter had the greatest concentration (table 2) [25]. Muscadine grapes are highly resistant to pathogenic infection and are native to areas of south-eastern United States, where there is a high incidence of fungal and bacterial diseases [25].

Table 2 about here

As resveratrol is synthesised in response to fungal attack [18], the amount of pathogenic infection can also have some bearing on its concentration within grape skin cells [15]. Jeandet *et al.* studied the extent of this relationship concluding that wines produced from grapes subjected to 40% or 80% infection by *Botrytis cinerea* (the fungus responsible for grey mould) had the lowest concentration of resveratrol [19]. In contrast, wines obtained from grapes infected by 10% *Botrytis cinerea* had the highest concentrations of resveratrol [19]. It was suggested in this study that these counterintuitive findings may be due to degradation of resveratrol by enzymes secreted by the fungus, in those grapes highly infected [19]. Thus only moderate *Botrytis cinerea* infection is required to provide maximal concentrations of resveratrol within wine.

The process of wine-making, namely vinification, greatly influences the concentration of resveratrol. It has been suggested that a longer maceration (softening through soaking) time increases the concentration of resveratrol in wines [10, 18]. Further research confirmed that maximal extraction of trans-resveratrol occurred 10 days following fermentation [24]. It is unsurprising that a longer time in contact with the grape skin results in higher concentrations of resveratrol in wine, as resveratrol resides in the skin, rather than the flesh of the berry [22].

Mechanisms of action

Resveratrol, isolated from *Polygonum cuspidatum* roots, had historically been used for remedial purposes in traditional oriental medicine against various diseases including gonorrhoea, athlete's foot, suppurative dermatitis, hyperlipidaemia and fungal skin diseases [20]. Table 3 summarises the diverse nature of its many other biological properties.

Table 3: about here

Few studies have researched the effects of resveratrol within the eye. The major actions on the eye include: anti-oxidant, anti-apoptotic, anti-tumourogenic, anti-inflammatory, anti-angiogenic and vasorelaxant. The following section will provide a review of the literature investigating each of these actions and their molecular mechanisms within the ocular structures.

Antioxidant activity

A positive correlation has been suggested between the antioxidant activity of red wine and its associated resveratrol content [44]. Compounds possessing antioxidant properties are able to combine with damaging free radicals, usually due to their structural composition, thereby stabilising them and preventing sustained oxidation. Oxidative stress is thought to be involved in the progression of several eye diseases including primary open angle glaucoma (POAG) [45] a major cause of worldwide irreversible blindness.

A recent study conducted by Luna *et al.* considered the therapeutic potential of resveratrol in combating the expression of glaucoma markers, in trabecular meshwork cells, resulting from chronic oxidative stress [46]. Their research demonstrated that resveratrol inhibited the increased production of intracellular reactive oxygen species (iROS) which in turn prevented the induction of the pro-inflammatory markers: interleukin-1a (IL1a), interleukin-6 (IL-6), interleukin-8 (IL-8), and endothelial-leukocyte adhesion molecule 1 (ELAM-1). In addition, chronic treatment with resveratrol prevented expression of the cellular senescence marker sa-β-galactosidase (sa-β-gal), typically induced by oxidative stress. The build-up of fluorescent pigments including lipofuscin, the end product of lipid peroxidation, and other carbonylated proteins were also found to be reduced. Luna *et al.* inferred that this was not due to decreased protein degradation since proteosomal activity was unchanged by resveratrol [46]. Furthermore, it was documented that resveratrol produced a significantly anti-apoptotic effect without having a detrimental effect on trabecular meshwork cell proliferation. Since oxidative stress is thought to cause damage to cells of the trabecular meshwork in the development of POAG, persistent treatment with resveratrol could prevent apoptotic cell death whilst maintaining cell proliferation.

The formation of age-related cataract is also associated with prolonged oxidative stress. While surgical removal is readily available for people in the developed world, the condition is a major cause of blindness in many poor and emerging countries. Although the exact mechanism of cataract formation has yet to be elucidated, it is thought that oxidation of proteins within the lens plays a crucial role in the pathogenesis. Sodium selenite acts similarly when injected into suckling rats, resulting in rapid cataract formation [47]. This represents a useful *in vivo* experimental model for the investigation of drug therapy in age-related cataract. Doganay *et al.* demonstrated a role for resveratrol in preventing selenite-induced cataract formation [47]. It was found that treatment with resveratrol caused an increase in the levels of reduced glutathione (GSH) in rat lenses and erythrocytes [47]. High levels of GSH can be isolated in the crystalline lens, where it serves to protect against damage by oxidants [48]. GSH levels have been shown to decline in age-related human and selenite-induced cataracts in rats, suggesting an essential role in preserving lens function [47]. Further to this, concentrations of malondialdehyde (MDA), a marker of lipid peroxidation in rat lenses and erythrocytes was significantly lower in resveratrol-treated rats [47]. These findings confirm the involvement of oxidative stress in the aetiology of selenite-induced cataract and a potential preventative role of resveratrol [47].

Resveratrol supplementation has recently been investigated in diabetic rats [49]. In this study, the supplemented rats were compared with diabetic and non-diabetic controls. Diabetes was induced by injection of streptozotocin 15 minutes after the prescription of nicotinamide in 12-hour fasted rats. Investigators reported that four months of oral resveratrol administration significantly alleviated hyperglycaemia, weight loss, enhancement of oxidative markers, and superoxide dismutase activity in the blood and retinas of diabetic rats. Resveratrol also suppressed the action of endothelial nitric oxide synthase (eNOS) in the eyes of diabetic rats. eNOS is associated with vascular neovascularisation and is actively involved in the inflammation and healing process in chronic diabetes [50]. The effect of resveratrol on vascular damage and VEGF induction has been assessed in the retinas of mice with induced diabetes. Diabetic changes such as increased vessel leakage, pericyte loss, and VEGF protein levels were locked by treatment with resveratrol [51].

In an another study, resveratrol was shown to inhibit endoplasmic reticulum stress, which contributes to retinal vascular degeneration, and in turn reduces ischaemia, reperfusion (I/R) and tunicamycin induced vascular degeneration [52]. Resveratrol also increases mitochondrial bioenergetics and protects against acrolein-induced cytotoxicity in human retinal pigment epithelial cells [53].

Anti-apoptotic activity

The retinal pigment epithelium (RPE) of the retina is subject to oxidative stress in the progression of proliferative vitreoretinopathy [54] and AMD [55]. It is this posterior blood-retinal barrier that facilitates the inward passage of metabolites and outward transport of waste material, thereby providing structural and nutritional support to the cells of the retina [56]. The lysosomal component of the RPE layer is responsible for the phagocytosis of the outer segments of photoreceptor cells, which are rich in polyunsaturated fatty acids (PUFAs) [57]. Phagocytosis in itself causes oxidative stress and further RPE cell damage is induced by peroxidation of the PUFAs, which are highly susceptible to free radical harm due to the presence of numerous double bonds [57]. Accumulation of lipofuscin within the RPE cells, as a result of incomplete degradation of these outer segments, also has deleterious consequences in retinal diseases such as AMD. Consequently, an abundance of reactive oxygen species (ROS) are produced which leads to degeneration of the RPE and dysfunction of the overlying photoreceptors.

Resveratrol has been shown to prevent programmed cell death of human RPE cells *in vitro*, induced by oxidative stress [58]. In this same study, researchers investigated the anti-proliferative effects of resveratrol, concluding that proliferation of RPE cells was reduced via inhibition of the extracellular-signal-regulated kinases one and two mitogen-activated protein kinase (ERK 1/2 MAPK) signalling cascade [58, 60]. Similar findings have also been reported in a variety of other tumour cell lines [59, 61]. The activity of ERK 1/2 (extracellular signal-regulated protein kinase) is a key participant in cell growth and proliferation of RPE cells [61]. King *et al.* suggested that the anti-proliferative effect of resveratrol is achieved by direct

inhibition of **M**APK/**E**RK **k**inase (MEK) (an upstream activator of ERK 1/2) and/or modulation of other initiator molecules upstream of MEK [58]. Laboratory studies have shown that sodium iodate can be used to mediate RPE cell death and that this is associated with elevated levels of ROS. Resveratrol was shown to protect RPE cells from sodium iodate [62].

Prevention of cell apoptosis is also very relevant in autoimmune-associated retinopathies. These are disorders of the eye in which auto-antibodies damage the retina and its components, causing a progressive loss of vision. As with AMD, age is a huge risk factor for the development of autoimmune retinopathies. Resveratrol has been reported to protect retinal cells from apoptotic death induced by auto-antibodies, *in vitro* (figure 3) [63]. The molecular pathology of autoimmune retinopathies is thought to involve an intracellular increase in Ca^{2+} ion concentration causing a loss of mitochondrial membrane potential [64]. This leads to a cascade of molecular events ultimately resulting in photoreceptor degeneration [64]. Anekonda and Adamus suggested that by inhibiting intracellular increase of Ca^{2+} ion concentration, resveratrol down regulated pro-apoptotic Bcl-2-associated X protein (BAX) concentration within the mitochondrion and cytoplasm of antibody treated retinal cells [63]. In addition, resveratrol was found to up-regulate anti-apoptotic proteins SIRT1 and Ku70 thereby suppressing BAX translocation into the mitochondria; a crucial step in mediating intrinsic apoptotic cell death [65].

Figure 3 about here

In another study by Kubota *et al.*, resveratrol was found to have a protective effect against light-induced retinal degeneration, a model used to investigate visual cell apoptosis [66]. This is thought to be an important contributing factor to the pathogenesis of diseases of a neurodegenerative nature including AMD and retinitis pigmentosa. Results revealed that light exposure caused an activation of retinal activating protein-1 (AP-1) and inhibition of SIRT1 activation, both of which were reversed by resveratrol [66]. Kubota *et al.* used terminal deoxynucleotidyl transferase deoxyuridine-triphosphatase (dUTP) nick end labelling (TUNEL) to detect DNA fragmentation as an indicator of apoptosis. After exposure to light, a significant

reduction in TUNEL positive cells was detected in the outer nuclear layer (ONL) of the retina, in the presence of resveratrol. Additionally, thinning of the ONL was significantly reduced. It was found that protection of the ONL by resveratrol significantly reversed the attenuation of the a- and b-wave as seen from electroretinography. Following light exposure, these protective effects were confirmed to last for at least 2 weeks.

The underlying mechanism thought to elicit these protective effects of resveratrol involves activator protein 1 (AP-1) a heterodimeric protein composed of c-fos and c-jun subunits [66]. AP-1 is responsible for the regulation of cell proliferation and apoptosis. In the results of this study, elevated levels of c-fos were located in the retinal nuclear extracts of mice exposed to light. Those treated with resveratrol, however, had significantly reduced levels of c-fos present. Thus resveratrol down regulated c-fos expression which in turn suppressed activation of AP-1, preventing programmed photoreceptor cell death which is otherwise upregulated upon light exposure.

Anti-tumourogenic activity

Resveratrol treatment has been shown to cause tumour cell death and regression in animal models of uveal melanoma by *promoting* apoptotic cell death [67]. Uveal melanoma is a malignancy of the uveal tract which consists of the choroid, ciliary body and the iris. Like AMD, uveal melanomas are much more common in people with fair skin and light irides [68]. One study has shown that resveratrol caused mitochondrial dysfunction in an animal model of uveal melanoma, similar to that found in autoimmune retinopathies. This involved release of cytochrome c and apoptogenic factors Smac/Diablo, subsequent activation of caspase-9 via APAF-1 and tumour cell death initiation because of caspase-3 activation [67]. Furthermore, following local injection of resveratrol, tissue was histologically examined and an increase in tumour cell death was found with adjacent tissues relatively unaffected by the treatment [67]. This suggests that higher concentrations of resveratrol targeted specifically to the site of the tumour could have a potentially greater therapeutic effect.

Pro-apoptotic pathways are also of significance in the management of tumorous growths. Resveratrol has been found to have chemopreventative activity in several cell lines, inhibiting tumour growth at various stages of progression [69]. One study demonstrated the ability of resveratrol to initiate apoptosis of human retinoblastoma cells [70]. Retinoblastoma is a rare tumour of the retina, but accounts for approximately 3% of all childhood cancers and is considered the most common primary intraocular malignancy among infants [68]. When unresponsive to radiation or chemotherapy, enucleation of the affected eye may be necessary to prevent metastasis of the tumour and subsequent complications [68]. Sareen *et al.* found that resveratrol initiated a time- and concentration-dependent growth inhibition of a retinoblastoma cell line by inducing cell cycle S-phase arrest and apoptosis [70]. Similarly to van Ginkel *et al.* [67], the findings of Sareen et al. established a primary role for mitochondria in resveratrol-induced programmed cell death [70].

Anti-inflammatory activity

There has been extensive research on the mechanism by which resveratrol achieves its anti-inflammatory action [71-74]. Inflammation is a key process in the pathogenesis of several ocular disorders including AMD [75] and DR [76]. DR is diabetic microangiopathy that affects the retinal vasculature due to poor metabolic control of blood glucose concentration. It results in progressive retinal damage due to ischemia and malfunction of the blood-retinal barrier, which can result in severe loss of vision. There are several molecular mechanisms thought to underlie an inflammatory response. Male rats injected with lipopolysaccharide were used to investigate the protective effects of resveratrol against oxidative stress [77] and inflammation [78]. It was reported that retinal leukocyte adhesion, a crucial event in mediating ocular inflammation, was significantly reduced in a dose-dependent manner following treatment with resveratrol. In addition to this, it was found that pre-treatment with resveratrol significantly suppressed the generation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), the principal biomarker of oxidatively modified DNA. Similarly, activation of a pro-inflammatory molecule known as nuclear factor (NF)-κB was found to be significantly suppressed, thereby inhibiting the pro-inflammatory signal transduction pathway downstream of oxidative stress [78]. Concentrations of 8-OHdG and NF-

κB were found to be upregulated in endotoxin-induced uveitis. NF-κB promotes the transcription of several inflammation-related target genes including intercellular adhesion molecule-1 (ICAM-1) and monocyte chemotactic protein-1 (MCP-1) [79], two molecules have been shown to be significantly suppressed following resveratrol application [78]. ICAM-1 and MCP-1 play an important role in recruitment [80] and subsequent adhesion [81] of leukocytes to vasculature endothelial cells during the inflammatory response, suppression of which may in part be due to the suppression of these molecules. An additional finding in this study involved sirtuin 1 (SIRT1) a histone deacetylase known to regulate aging [82]. Resveratrol has already been identified as an activator of SIRT1 extending the lifespan of several species including yeast, rodent and others [83-86]. Kubota *et al.* showed that an otherwise reduced RPE-choroidal activity of SIRT1, typically resultant of the inflammation present in endotoxin-induced uveitis, was significantly inhibited upon oral administration of resveratrol [77]. These results were consistent with the decline in NF-κB activation and decreased RPE-choroidal degradation of IκB-α, a process required for the translocation and subsequent activation of NF-κB. The authors proposed a dual mechanism by which resveratrol achieves its anti-inflammatory effect in endotoxin-induced uveitis: firstly as an anti-oxidant and secondly as a potent activator of SIRT1 (figure 4).

In 2010, Kubota *et al.* found that SIRT1 activity is not only down regulated in conditions involving inflammation but also during exposure to light [66]. SIRT1 activity was significantly improved following resveratrol treatment, in agreement with their previous findings [78]. Thus, Kubota *et al.* concluded that SIRT1 activation, along with AP-1 deactivation led to prevention of photoreceptor cell death and subsequent protection against light-induced retinal degeneration [66].

Figure 4 about here

SIRT1 activation is also implicated in neuroprotection [87]. Optic neuritis, a commonly encountered complication of multiple sclerosis (MS) is an inflammatory ocular disease characterised by demyelination of the optic nerve. Subsequent axonal damage leads to a progressive loss of visual function in the affected eye. Schindler *et al.* investigated the

neuroprotective role of SIRT1 activation on an experimental autoimmune encephalomyelitis (EAE) animal model of MS, using two chemically distinct SIRT1 activators [88]. One of these included a proprietary formulation of resveratrol, called SRT501. The investigators found that SRT501 reduced retinal ganglion cell (RGC) loss in a dose-dependent manner. Furthermore, SRT501 was found to provide long-standing RGC neuroprotection with two episodic declines in clinical signs of EAE over a period of 30 days following immunisation with proteolipid protein peptide. This was in contrast to one decline seen at 14 days with another SIRT1 activator investigated in the same study. It seemed that SRT501 showed similar efficacy in preserving axon function and preventing RGC loss when administered as a single dose or multiple dose 11 days after immunisation [67]. However, repeat administration of SRT501 did not prevent the development of EAE nor optic nerve inflammation in the animal model of MS [88]. This suggests that this resveratrol formulation targets RGC loss and could have therapeutic benefits when used in combination with immunomodulatory therapies. Additionally, SRT501 toxicity to photoreceptors and retinal function was investigated. No change in retinal thickness or RGC quantity was detected in control eyes following treatment, demonstrating that SRT501 was not toxic at the concentrations applied. Schindler *et al.* proposed that the neuroprotective benefits of SRT501 were due to its upregulation of SIRT1 activity. This was confirmed by blockade of RGC protection by a SIRT1 inhibitor, sirtinol, at a concentration non-toxic to the retinal layers [88].

More recently, SIRT-1 activators have been reported to reduce oxidative stress and promote mitochondrial function in neuronal cell [89,90]. SIRT1 activators can prevent cell loss and mediate neuroprotective effects during optic neuritis via these mechanisms [89]. Moreover, SIRT-1 activators, like resveratrol, have the potential to preserve neurons in other neurodegenerative diseases that involve oxidative stress. Another study also using EAE in the mouse model similarly showed that resveratrol can prevent neuronal loss in MS [91]. The neuroprotective effects were reported to occur without immunosuppression, which might suggest a potential additive benefit of resveratrol in combination with anti-inflammatory therapies for MS. SIRT1 is also associated with reduced oxidative stress following traumatic

injury and has been shown to reduce RGC loss in mice following induced optic nerve crush injury [92].

The neuroprotective effect of resveratrol on experimental retinal ischaemic injury has also been investigated [93]. In this study, resveratrol or saline was administered to adult rats via intraperitoneal injection daily for five days. On the third day retinal ischaemic injury was induced in the rats by elevation of intraocular pressure for 45 minutes. Retinal function was measured using one week after the induced ischaemic injury and was compared with readings taken prior to resveratrol administration. Marked thinning of the inner retinal layers was observed following the ischaemic injury, but this was reduced in those rats receiving resveratrol.

In a study of the potential neuroprotective effects of resveratrol against photoreceptor cell death in a rodent model of retinal detachment, it was found that resveratrol up-regulates the FoxO family and blocks Caspase 3, 8 and 9 activation. Investigators concluded that resveratrol may have a role in preventing vision loss in disease characterised by photoreceptor detachment [94].

Inflammation induced by chronic hyperglycaemia is linked to the pathogenesis of DR [95]. Such conditions lead to gradual RPE cell degeneration and resultant degradation of the blood-retinal barrier [96]. Subsequent loss of central vision is the expected outcome, since integrity of the blood-retinal barrier is compromised and normal visual function depends on the functionality of this ocular component [97]. Hyperglycaemic conditions cause a decreased expression of connexin (Cx) 43 [98] resulting in reduced gap junction intercellular communication [96]. Gap junctions are intercellular connections formed by connexin proteins that allow direct communication between the cytoplasm's of adjacent cells, allowing the passage of various small molecules and ions [96]. Abundantly expressed at the protein level in endothelial cells of the retinal vasculature, Cx43 is thought to sustain blood-retinal barrier integrity and normal intercellular communication [96]. Losso *et al.* demonstrated the ability of resveratrol to inhibit RPE cell inflammation, caused by hyperglycaemia *in vitro* [96]. They found that hyperglycaemia generated the expression and downstream upregulation of several inflammatory molecules.

Treatment of the cells with resveratrol significantly inhibited the accumulation of these molecules, including vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), cyclooxygenase-2 (COX-2), interleukin (IL) 6 and IL-8 in a dose-dependent manner. Activity of protein kinase C (PKC) β, an enzyme that up regulates VEGF in hypoxic conditions further contributing to blood-retina barrier degradation, was reduced in the presence of resveratrol [96]. Resveratrol was also found to prevent hyperglycaemic down regulation of Cx43 protein in RPE cells thereby enhancing gap junction intercellular communication, crucial to the integrity of the blood-retinal barrier [96]. The mechanisms leading to hyperglycaemic inflammation and sites of intervention by resveratrol are shown in figure 5.

Figure 5: about here

Anti-angiogenesis activity

Prevalence of abnormal retinal and choroidal angiogenesis among ocular disorders is comparable to that of inflammation, and is a leading cause of blindness in diseases such as DR and AMD. A report on three cases of resveratrol-based supplementation in octogenarians with AMD showed a short term effect similar to that found with anti-VEGF treatment. This included anatomical restoration of retinal structure, improved RPE function and a suggestion of improved choroidal blood flow [100].

Macular telangiectasia (MT) is another disease of the retina which, similar to AMD, is characterised by abnormal neovascular proliferation of the retinal vasculature surrounding the fovea [101]. This is associated with gradual photoreceptor degeneration [102]. MT can be distinguished from AMD by its absence of choroidal neovascularisation, typically present in AMD [102]. However, there is little known about the mechanisms surrounding the pathogenesis of this disease and few treatments are available. Hua *et al.* used the mouse model with an insertion mutation in the gene encoding for the very low-density lipoprotein receptor (VLDLR) to investigate the therapeutic potential of resveratrol [101]. The mice displayed patches of retinal neovascularisation without preliminary damage to the RPE and photoreceptor cell death, similar

signs to that found in people with MT. Findings confirmed that neovascular lesions identified following VLDLR mutation were, at least in part, due to the increased expression of VEGF. This is supported by the suggestion that focal retinal vessel leakage in MT is due to upregulation of VEGF [103] and that intravitreally injecting these patients with ranibizumab (a VEGF inhibitor) decreases the progression of abnormal angiogenesis [101]. Results from this study demonstrated a reduction in neovascularisation in VLDLR mouse retinas upon oral administration of resveratrol by inhibiting VEGF expression [101]. This was the case when resveratrol was administered before and following the initiation of neovascular lesions. Furthermore, resveratrol reduced migration and proliferation of retinal endothelial cells as triggered by other stimulus pathways. This indicates that the anti-angiogenic effects observed were independent of the VLDLR mutation. This is consistent with results from a previous study, revealing that resveratrol modulates pathological angiogenesis via an elongation factor-2 kinase-regulated pathway [104].

Khan *et al.* found that resveratrol prevented pathologically aberrant injury-induced angiogenesis by a novel SIRT1-independent pathway, involving eukaryotic elongation factor-2 (eEF2) (figure 6) [104]. This protein plays a crucial role in protein synthesis, specifically in the ribosomal translocation of the polypeptide chain. It is known that eEF2 is inactivated by EF2 kinase phosphorylation, an enzyme which is activated by resveratrol via phosphorylation of a serine residue at location 398 by AMP-activated protein kinase (AMPK) [105]. This leads to cell cycle arrest thus inhibiting endothelial cell proliferation and migration, two crucial events in the progression of angiogenesis [104]. By pharmacologically inhibiting AMPK, Khan *et al.* further validated these results by demonstrating significant reversal of the observed effects on vessel growth [104].

Figure 6 about here

The effect of resveratrol supplementation on experimental corneal alkali burns has been investigated [106]. A corneal alkali burn was administered in 62 eyes of 31 male rabbits.

Resveratrol was administered to both eyes via subconjunctival injection for seven days. Corneal photographs and inflammatory index scores indicated that Resveratrol had no effect on corneal neovascularisation.

Aberrant growth of new vessels also predominates as a pathological feature of retinopathy of prematurity (ROP). The leading cause of blindness among prematurely born infants, ROP is a proliferative retinopathy affecting prematurely born infants. Particularly at risk are those that are less than 1300 grams in weight and/or less than 30 weeks gestation. The disease can be divided into several stages ranging from a mild type of demarcation line to a severe total tractional retinal detachment and potential blindness. ROP develops upon exposure of immature retinas to high ambient levels of oxygen. Once the infant is removed from the high levels of oxygen, abnormally fast growth of new vessels occurs leading to fibrosis and scar tissue in the retina which subsequently detaches. As with MT there is an upregulation of VEGF in ROP, secreted mainly by the retinal astrocytes and Muller cells [107]. During the initial exposure to hyperoxia, however, levels of VEGF and other proteins including insulin-like growth factor (IGF)-1 and hypoxia inducible factor (HIF) are low. IGF-1 and HIF are factors that affect the activity of VEGF. The former allows VEGF to maximally stimulate blood vessel proliferation and the latter is a transcriptional factor for VEGF. Once the infant is supplied with normal oxygen levels, regions of the retina receiving a lack of oxygen become hypoxic. This retinal hypoxia causes IGF-1, HIF and VEGF stimulation. VEGF promotes endothelial cell proliferation and pathological growth of new vessels. In a study conducted by Kim *et al.*, *in vivo* and *in vitro* oxygen-induced retinopathy models were used to define the effectiveness of resveratrol as a potential treatment [107]. They found that the expression of inducible nitric oxide synthase (iNOS) antibody and mRNA was increased, whereas a reduction in eNOS and neuronal NOS (nNOS) was evident upon resveratrol treatment. NOS catalyses the synthesis of nitric oxide (NO), a molecule that prevents platelet aggregation and promotes vasodilation. This research suggested a role for NO-mediated pathways in the protection of retinas of pre-term babies [107].

Vasorelaxant activity

It has been demonstrated that resveratrol can induce vascular relaxation [100]. With its ability to inhibit angiogenesis [99, 103] this makes it an ideal candidate for disorders of the eye associated with impaired ocular perfusion, such ROP and DR. Vasorelaxation of small-diameter retinal arterioles was observed in a dose-dependent manner, when treated with resveratrol [108]. The research showed that disruption of the vasculature endothelium by denudation, decreased but did not eliminate vasodilation elicited by resveratrol, suggesting the presence of endothelium-dependent and independent mechanisms. Although reports have suggested a predominantly endothelium-dependent component, this study proposed that the endothelium-independent mechanism bares great significance for diseases in which the endothelium function is impaired, for example clinical obesity and juvenile onset diabetes. This study established that resveratrol-induced vasodilation was prevented in part by N^G-nitro-L-arginine methyl ester (L-NAME) an NOS antagonist, similar to the consequences of vessel denudation. This suggested a role of NO in the resveratrol-induced endothelium-dependent aspect of vasodilation. To confirm this finding Nagaoka *et al.* blocked the synthesis of other molecules predominantly involved in vasodilation, namely prostacyclin and cytochrome P450 metabolites [108]. The resveratrol response was unaffected by this, thus supporting the involvement of NO alone in the endothelium-dependent component of vasodilation [109]. The vasodilatory effects observed in this study were reduced by specifically inhibiting the ERK pathway, similar to the decrease produced by L-NAME and vessel denudation. This suggests the potential involvement of the ERK/MAP kinase signalling pathway in the vasodilation caused by resveratrol, mediated by cyclic guanosine monophosphate (cGMP). The endothelium-independent component of resveratrol-induced vasodilation was found to be primarily mediated by the activation of big potassium (BK) calcium channels (calcium activated potassium channels) present in the smooth muscle of the vasculature. This was proven by iberiotoxin, a calcium channel antagonist, inhibiting the vasodilatory response. Therefore Nagaoka *et al.* were successfully able to provide evidence of two independent mechanisms by which resveratrol produced vasorelaxation and thus vessel diameter dilation within isolated retinal arterioles [108].

Discussion

Resveratrol has been reported to have beneficial ocular and systemic beneficial effects. Literature on the association between resveratrol and the eye was examined with emphasis on potential mechanisms by which resveratrol is thought to prevent the progression of ocular diseases, particularly those that are degenerative and responsible for the prevalence of severe sight loss.

Age-related macular degeneration and DR predominate in Western populations, mainly due to increased life expectancies in developed countries. AMD is the leading cause of blindness among older people, aged typically over 65 years while DR is the most common cause of vision loss among those of a working age. In contrast, conditions such as glaucoma and cataracts are common causes for blindness in less economically developed countries. A lack of cure for these diseases, amongst many others, means that their progression can only be monitored and delayed usually by pharmacological means or surgical intervention.

Development of conditions including AMD, DR and glaucoma rely heavily on a positive family history. However, treatments used to manage these conditions are only implemented once a positive diagnosis is made. This is not an ideal approach for the pre-disposed person who may benefit from early preventative measures, delaying the onset rather than the progression of the disease. A vast amount of scientific research has been conducted into natural compounds proposed to be beneficial in protecting the eye from developing sight threatening diseases. Some of these compounds, such as lutein and ginkgo biloba, have been formulated into nutritional supplements. However, have yet to become widely recognised among the public and practitioners. Patients trust their healthcare providers and as such, practitioners have a responsibility to educate themselves and their patients about *all* preventative treatment options where clinically applicable.

Limitations of previous studies and direction for future research

Although the research conducted thus far has provided a great deal of valuable understanding of the molecular mechanisms by which resveratrol produces its favourable effects on the eye, there are many factors limiting firm conclusions. In all of the literature reviewed, there has been no attempt to investigate a wide range of resveratrol concentrations, in effort to define lethal dose. Using concentrations of 50, 100 and 200μm, Sareen *et al.* found that the latter had the highest potency in retinoblastoma cell growth inhibition, with IC_{50} of 170 μm [70]. Similarly, another study demonstrated doses of resveratrol between 2 and 50mg/kg caused inhibition of uveal melanoma cell growth, with maximal effect at the highest dose [67]. The range of concentrations was not extended in these studies to find the dose at which resveratrol surpassed its desirable effect. This is essential in determining the range of concentrations over which resveratrol produces its beneficial effects optimally and yet remains safe to the subject.

None of the literature reviewed used human subjects for experimentation. Instead, studies were conducted *in vitro* or using animal models. Whilst animal studies are a crucial aspect of pharmacological research, the overall effects of resveratrol observed on animals can greatly differ from that on humans. Human studies are required before making an informed conclusion on whether resveratrol is as beneficial as has been suggested. Furthermore, values such as LD_{50} and IC_{50} can differ between species due to physiological differences. Hence, effective concentrations of resveratrol in animals may vary in humans.

Serum concentrations of orally-administered resveratrol have been determined as low [67]. This is analogous with reports of low systemic bioavailability of the phytoalexin [109]. In one study it was found that locally injecting resveratrol, adjacent to the site of the uveal melanoma, had a greater effect in reducing tumour size [67]. This demonstrates that local administration can increase the resveratrol concentration at the precise site its action is required, therefore enhancing its beneficial activity. Further research is necessary to determine the most efficient method of resveratrol delivery, as oral administration may not be the most effective choice for disorders of the eye.

Since resveratrol has been well documented for its beneficial protective mechanisms, it has been largely believed to be the active compound within red wine. However, as discussed there reside other red wine polyphenols that have received comparatively less interest. The literature reviewed has considered the effects of isolated resveratrol rather than within red wine, where interactions with other chemicals could potentially influence the effectivity of the stilbene derivative. Polyphenols have been recognised as antioxidants [13], thus biological activities of resveratrol may be enhanced in combination with these compounds, or maybe even reduced. This remains to be investigated.

Clinical implications

Alcohol is an obvious constituent of red wine and it has been established that alcohol itself is able to increase high density lipoprotein (HDL) concentration [2]. Thus it may be argued that its content is partially responsible, along with resveratrol, for some of the benefits observed by red wine consumption. However, alcohol is toxic when heavily consumed as depicted by the J-shaped curve [2]. A recent UK Government campaign has received much media attraction as it warns that two glasses of wine a day, which may be considered as 'moderate' by many, can lead to a three times greater risk of contracting mouth cancer [110]. In addition, alcohol consumption is often associated with smoking, which in itself is a risk factor for several cancers and eye diseases including AMD.

Alcohol consumption is commonly contraindicated with several medications and systemic conditions. Many patients frequently seeking primary eye care are older, and with age various systemic and ocular degenerative diseases, become more common. A proportion of patients will not benefit from the positive effects that occur with wine consumption because alcohol consumption is contra-indicated. Furthermore, underage drinking is strictly prohibited and individuals under the age of 18, suffering from any ocular disease which may be ameliorated by red wine consumption, are forbidden from doing so. At present research is inconclusive in terms of the benefits of red wine. With all this in mind, it is questionable whether it would be responsible for a health care professional to recommend or promote red wine consumption.

What is likely to be safer is the use of resveratrol supplements. This would be far more appropriate to suggest in a clinical environment but also since most of the literature demonstrates the beneficial actions of resveratrol alone, isolated from any other chemical influence. There needn't be any age restriction or contraindication in the absence of alcohol, and it may be considered acceptable in the treatment of diseases such as ROP [108].

Conclusion

This review highlights evidence which suggests that resveratrol may have potential in the treatment of several ocular diseases. However, while there are many studies indicating plausible biological mechanisms using animal models and *in vitro* retinal cells there is a paucity of human research. We note that there are many nutritional supplements containing resveratrol as a single component or in combination with other nutrients that are promoted as being of benefit in long term eye health. However we urge caution in that evidence base for the use of resveratrol in the management of ocular diseases needs to be improved before any recommendations can be made for the use of resveratrol as an ocular supplement.

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Figure captions

Figure 1: Chemical structure of a simple phenol (chemical formula: C_6H_5OH). The structure characteristically comprises a benzene ring associated with a hydroxyl group

Figure 2: Chemical structure of cis- and trans-resveratrol [23]. The structures comprise of two phenol groups joined by a methylene bridge.

Figure 3: Molecular mechanism by which resveratrol is suggested to prevent antibody-induced retinal cell apoptosis [64]. Resveratrol is shown to prevent intracellular increase of Ca^{2+} ion concentration, otherwise induced by proteins that have been activated by antibodies entering the cell via endocytosis. This prevents mitochondrial activation of cytochrome c (cyt c) and subsequent increase in caspase-3 activity via caspase-9 and APAF-1. SIRT-1 and Ku70 are upregulated by resveratrol, therefore preventing the entry of Bax into the mitochondria. Both actions inhibit cell death initiated by caspase-3.

Figure 4: Proposed mechanisms through which resveratrol prevents ocular inflammation in endotoxin-induced uveitis (Adapted from [78]). The diagram depicts a dual role of resveratrol as an anti-oxidant and a SIRT1 activator. Dashed arrows represent pathways inhibited by resveratrol.

Figure 5: Inhibition of hyperglycaemia associated inflammation by resveratrol. The normal events leading to inflammation are shown schematically. Dashed arrows represent the multiple sites of inhibition by resveratrol, causing disruption to these pathways and subsequent prevention of inflammation induced by chronic hyperglycaemia.

Figure 6: Diagrammatic representation of angiogenesis inhibition mediated by resveratrol [104]. Resveratrol indirectly activates eEF2 kinase, via AMPK. This causes inactivation of eEF2, preventing endothelial cell proliferation and migration.

Table captions

Table 1: Categorisation of polyphenols (Adapted from [13]). Two types of polyphenols are found within red wine: flavonoids and non-flavonoids. These are further divided into subtypes, shown in italics. Examples of each of the subtypes are given in brackets.

Table 2: A comparison of resveratrol content in red wines produced by three varieties of grape species [25]. Measurements were obtained by gas chromatography analysis (average mean values given in parts per million).

Table 3: Some of the main biological properties of resveratrol.

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Flavonoids	**Non-flavonoids**
Flavonols (Quercetin & myricetin)	*Stilbenes* (Resveratrol)
Flavan-3-ols (Catechin, epicatechin & tannins)	*Hydroxycinnamates* (Caffeic, caftaric & coutaric acids)
Anthocyanins (Peonidin & cyanidin)	*Hydroxybenzoates* (Gallic acid)

Table 1: Categorisation of polyphenols (Adapted from [13]). Two types of polyphenols are found within red wine: flavonoids and non-flavonoids. These are further divided into subtypes, shown in italics. Examples of each of the subtypes are given in brackets.

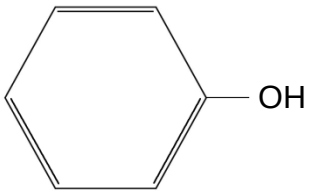
Species	Cis-resveratrol content	Trans-resveratrol content
V. rotundifolia	21.2	9.1
V. vinifera	3.3	3.6
V. labruscana	2.7	2.6

Table 2: A comparison of resveratrol content in red wines produced by three varieties of grape species [25]. Measurements were obtained by gas chromatography analysis (average mean values given in parts per million).

Property	Key References
Anti-microbial	26, 27
Chelation of copper	28
Anti-oxidant/ free radical scavenging	29, 30
Oestrogenic activity	31, 32
Inhibition of lipid peroxidation	33, 34
Anti-cancer	35
Inhibition of platelet aggregation	36, 37
Anti-inflammatory	38, 39
Vaso-relaxant	40, 41
Inhibition of eicosanoid synthesis	42
Modulation of lipid metabolism	43

Table 3: Some of the main biological properties of resveratrol. Corresponding references are provided for further insight.

Figures Bola et al Red Wine and the Eye: Activity and Molecular Mechanisms



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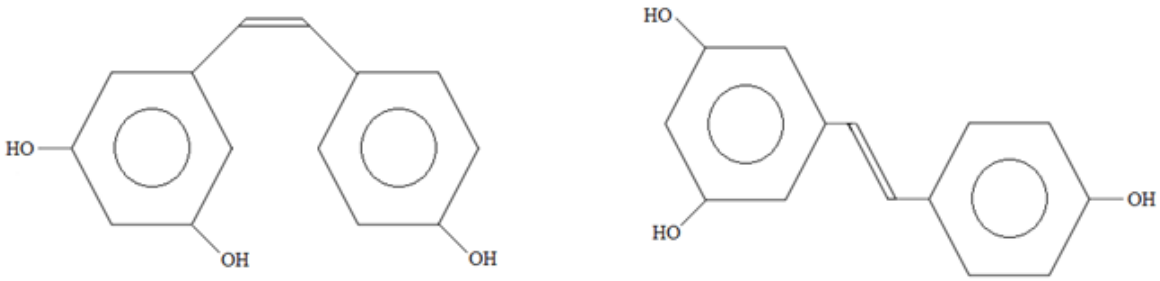
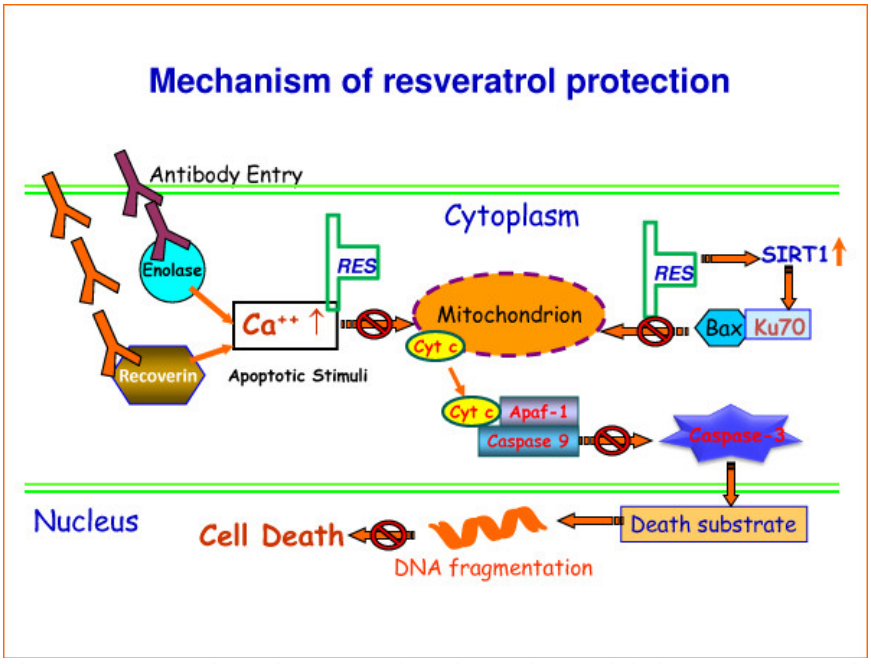


cis-resveratrol trans-resveratrol



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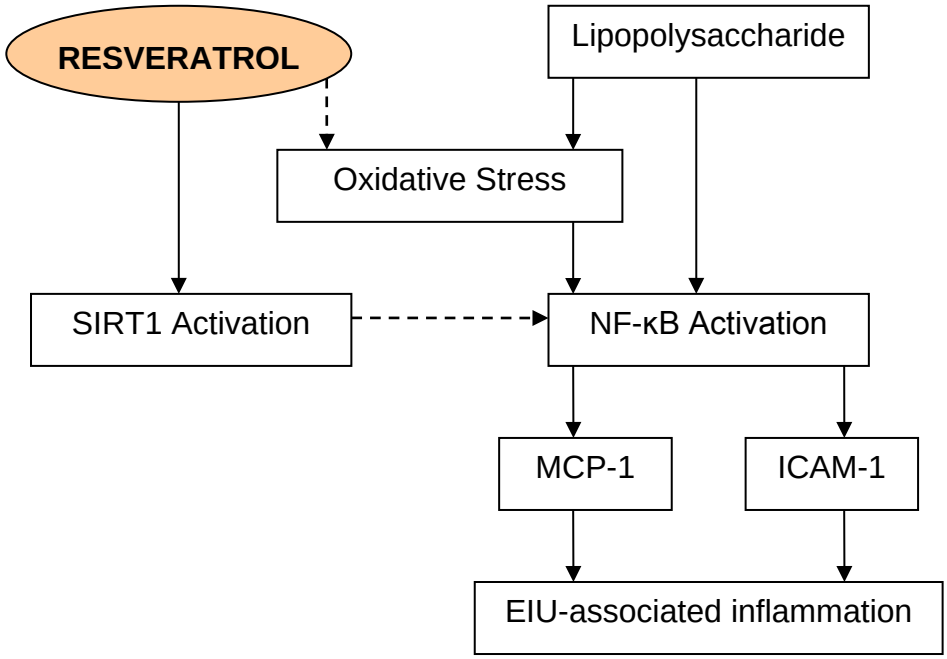

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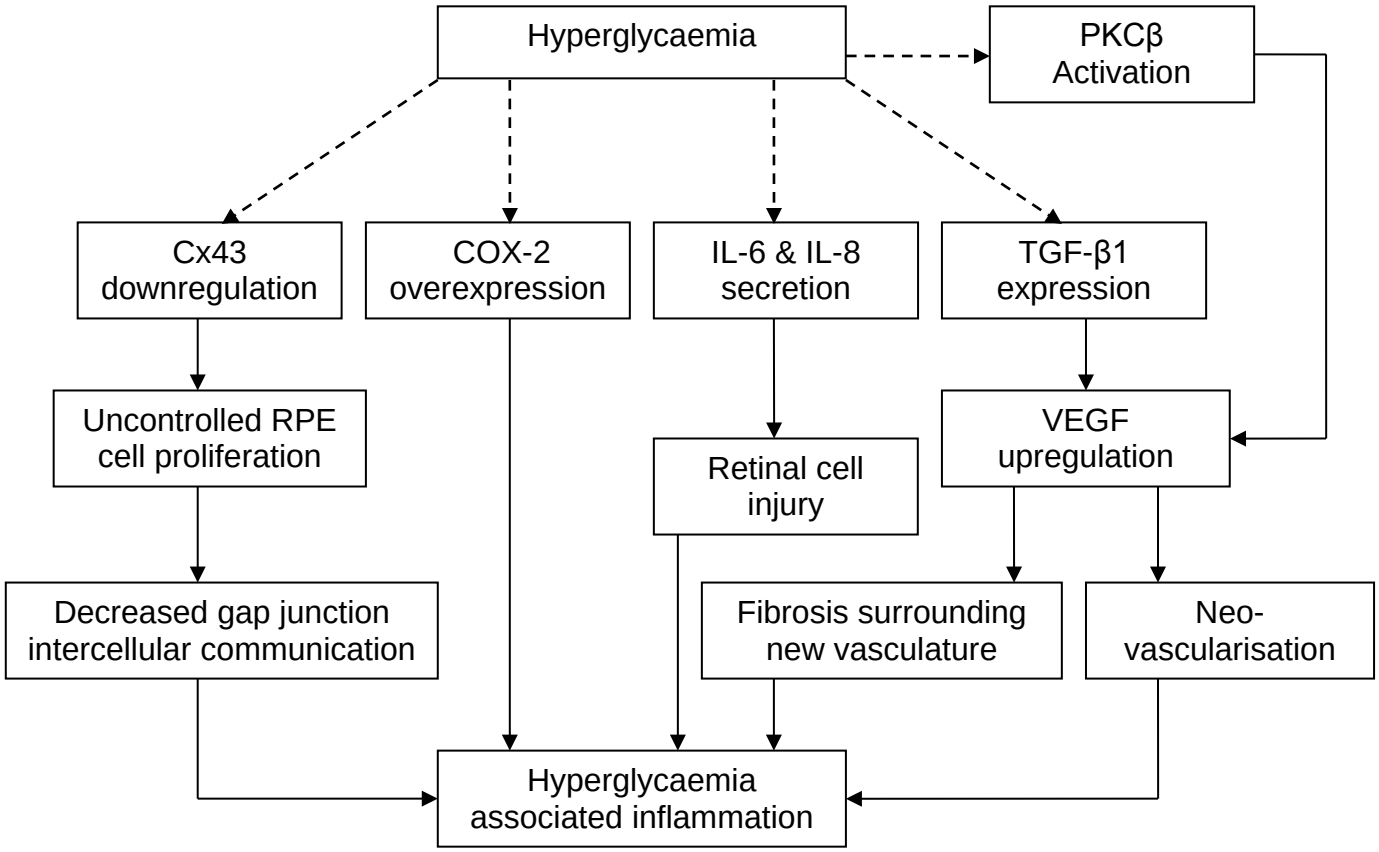

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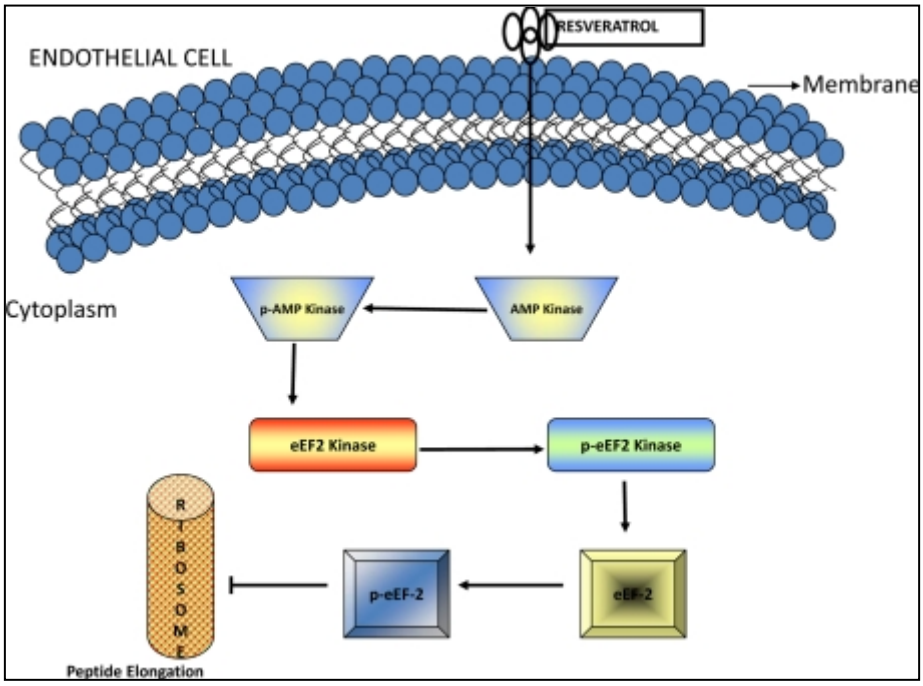

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