The Benefits of Calorie Restriction and Calorie Restriction Mimetics as Related to the Eye

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Abstract: The effects of calorie restriction without malnutrition seem to possess many beneficial effects in numerous disease states. Recently, studies related to calorie restriction mimetics that biochemically mimic the effects of calorie restriction are also becoming increasingly popular. Both calorie restriction and calorie restriction mimetics trigger an adaptive response reminiscent of mild-stress or low-dose toxic response, which is frequently referred to as hormesis in the toxicology literature. Although some benefits of calorie restriction and calorie restriction mimetics have been studied, the role of hormesis-related pathways in the eye has not been given a special attention. This review will present the current literature on calorie restriction and calorie restriction mimetics as related to most prominent eye diseases and provide insights on the therapeutic role of hormesis in eye diseases.

INTRODUCTION

The health benefits of calorie restriction (CR) have been known to the scientists for decades. In the recent literature, CR or dietary restriction has been generally defined as consumption of nutritious diet that is 40% less in calories compared to *ad libitum* diet. In addition to providing protection against numerous deadly diseases such as cancer, neurological disorder, and obesity, CR is the only reliable treatment that extends life span or causes healthy aging consistently in a multitude of organisms ranging from bacteria to monkeys [1-4]. The extent to which CR extends human life span, however, is still largely unknown. The most frequently touted effect of CR has been its influence on creating a mild stress in the organism and a typical up-regulation of adaptive mechanisms involving stress proteins accompanied by elevated defense or survival molecules [5-7]. This response is similar to the expected hormetic response [8]. According to the theory of "hormesis," toxins and pollutants generally show biphasic dose response, where a low dose of toxin triggers a positive, adaptive stress response, which may help an organism sustain much higher levels of toxins which otherwise cause harmful effects [7-12].

Although CR can benefit human health and extend longevity, its success depends on determined change in human behavior—willingness to mildly starve! Ironically, the current calorie consumption trend in the United States is just the opposite, with weight gain and obesity expected to increase in the coming decades [13]. Realizing that it is difficult to influence public calorie consumption patterns, scientists have been contemplating alternative ways to accrue the benefits of CR without actually suggesting restriction of calorie intake. Such a strategy is made possible because a large number of plant-derived chemicals or phytochemicals can mimic the effect of CR. These phytochemicals are referred to as CR mimetics (CRMs) [14, 15]. CRMs at low doses function like toxicants and trigger the adaptive response in the organism [8-10, 12]. Fruits, nuts, vegetables and herbs together offer an estimated 4000 different kinds of flavonoids [16-18] that may have been evolved to fight diseases and insect attack [14], but at proper dosage levels may be extremely beneficial to human health. The popularity of herbals can be attributed to the public belief that herbs are naturally safer than synthetic drugs [19]. Experts believe that one in three Americans uses herbal supplements, with the consumption level much greater among women [20, 21], patients undergoing surgery [22], and the elderly. Because herbals seem to embody both medical and marketing benefits, scientists are excited about their prospects as suitable alternatives to the CR.

Both CR and CRMs affect a common pool of biochemical pathways that are implicated in organism's survival and longevity. Specifically, the following two biochemical pathways and associated proteins have shown much promise in the last decade: (1) Sirtuin pathway [5, 6, 23-25], and (2) Kelch-like ECH-associated protein 1 (Keap1)/nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway, simply referred to as the Keap1/Nrf2/ARE pathway [26]. While anti-aging sirtuin pathway is implicated in an organism's longevity [25], ARE pathway is known to trigger the upregulation of cytoprotective genes essential for cell survival [26]. Emphasis in the eye literature addressing these specific pathways is limited and only currently emerging literature seems to provide some initial insights into the roles of these pathways in the eye. This review will focus on the benefits of CR and CRMs as related to the eye, particularly recognizing the potential hormetic pathways essential for survival and longevity.

DISEASE AND ANATOMY OF THE EYE

In epidemiological and nutritional supplement studies, traditionally known antioxidants (vitamins A, C, E) and carotenoids (lycopene, lutein, zeaxanthin) appear to have yielded a varying, but inconsistent degree of effectiveness in minimizing the damage caused by age-related eye diseases

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such as cataract, age-related macular degeneration, and glaucoma [27-29]. A broad range of herbal compounds has also been found beneficial to delaying age-related eye diseases [27-29].

The human eye, frequently referred to as the window to the mind, suffers from many diseases that may ultimately cause blindness. According to 2000 US Census, nearly 1 million Americans older than 40 years of age were blind, with an additional 2.4 million suffering from low vision [30]. The leading causes of blindness and low vision included age-related macular degeneration (ARMD), cataract, glaucoma, diabetic retinopathy, and other diseases [30]. Inflammation in the eye including uveitis causes about 10% of blindness in the United States. The blindness in the US is projected to increase 70% by 2020, largely due to the aging population [30]. Because most debilitating eye pathologies are age-related, the treatments that modulate aging pathology or age-related biochemical pathways are expected to contribute significantly for developing suitable treatments for age-related diseases.

The diseases and pertinent treatments of the eye can be better understood by knowing the basic anatomy of the eye, so a brief description of the human eye anatomy will be presented below. The anterior portion of the eye constitutes cornea, anterior chamber, iris, and the anterior ciliary muscle. The eye lens divides the anterior chamber from the posterior segment, which includes the posterior ciliary body, vitreous fluid, retina that surrounds the vitreous fluid, choroid, and the outer sclera. The iris, ciliary body and choroid together form the middle pigmented layer of the eye called the uvea. The cross-section of different layers of the eye from retina through sclera show complex layers of cells that perform specialized functions. The retina is a thin layer of transparent nerve tissue with at least five distinct types of nerve cells: the outermost photoreceptor cells (rods and cones), horizontal cells, bipolar cells, amacrine cells, and ganglion cells. The photoreceptor cells receive the light and transmit it to the other nerve layers all the way down to the ganglion cell layer. The retinal ganglion cells forming the optic nerve transmit the light signals to the brain. Immediately adjacent to the outer photoreceptor segment is a single layer of cells called retinal pigment epithelial cells (RPE), which among other things perform crucial phagocytosis function to recycle the debris that was created by the dead photoreceptor cells. The nourishment and oxygen supply to the retina is provided by the choroid, which in turn has four layers: the inner Bruch's membrane, choriocapillaris with small capillary vessels, a layer with medium-sized blood vessels (Sattler's layer), and the outermost layer with large vessels (Haller's layer). The outer most protective layer of the eye is called sclera.

CALORIE RESTRICTION

CR studies elsewhere in the body have re-emerged with a renewed interest in the recent years, especially in research related to cancer, obesity, and the brain. Eye research, however, appears to have fallen short in identifying the emerging beneficial molecular pathways of CR to sight-related diseases. Only a limited literature is available on the beneficial effects of CR against cataract, ARMD, and glaucoma, and this evidence is discussed below.

Benefits of CR in Cataract

Several animal studies have shown the beneficial effects of CR diet against cataract, the development of clouding in the crystalline lens of the eye leading to a progressive increase in the lens opacity associated with the loss of transparency [31, 32]. Calorie restriction (at 60-79% of *ad libitum,* AL, intake) in Emory mice, delayed the onset, formation, progression, and accumulation of cataract, besides extending the median life span of the animals by 40% [33-36]. Interestingly, the delayed accumulation of cataract did not correlate with the level of antioxidant enzymes [37]. In B6D2F1 mice (C57BL/6 x DBA/2), CR diet attenuated the decline of proliferative capacity of lens epithelial cells (LE) in older mice [38], and in old, CR mice LE cells were more resistant to H_2O_2-induced cell damage than LE cells in old, AL mice [39]. Of the two rat strains (Brown Norway & Fischer 344), and three mouse strains (C57BL/6, (C57BL/6 x DBA/2)F1, (C57BL/6 x C3H)F1), CR diet extended life span in all strains, and delayed the first appearance and subsequent severity of cataract in four dark-eyed strains, but not in albino, Fischer 344 rats [40]. Further, in Brown Norway rats, CR diet attenuated age-related shortening of telomeres in LE cells [41]; retarded age-related degeneration of lens by reducing oxidative stress in the lens [42]; and, prevented age-related decline in glycolytic enzymes, molecular chaperones, and αB-crystallin, a lens protein [43]. In Wistar rats, 50% food restriction and 75% protein restriction lowered β-and γ-crystallin aggregation and the chaperone activity of α-crystallin was improved by 50% vitamin restriction [44]. These studies clearly suggest that CR diet enhances an animal's longevity irrespective of the pigmentation, and protection against cataract occurs only in pigmented animals, but not in albinos. This anomaly in albino rodents may be related to their greater susceptibility to light-induced damage than the normal brown rodents.

Benefits of CR in Age-Related Macular Degeneration

ARMD is the leading cause of blindness in white Americans and it accounts for 54% of all blindness [30]. In ARMD, the increased degeneration of macula, a 5.5 mm area around fovea, occurs with aging and more severely after 60 years of age. One of the hallmarks of ARMD includes the extracellular deposition of misfolded and aggregated proteins, drusen bodies, beneath RPE and Bruch's membrane. Although age is the strongest risk factor for ARMD, several other systemic risk factors have also been identified: hypertension, smoking, and family history [45]. The degeneration and death of photoreceptor cells in the macula leads to the loss of central vision, then a progressive loss of peripheral vision. In most severe case, the in-growth of blood vessels from choroid rupture the Bruch's membrane and move into the sub-RPE and sub-retinal space leading to choroidal neovascularization [45]. Surprisingly, compared to CR studies in cataract, CR-related studies in ARMD are less conclusive. Given the fact that CR effects possess sirtuin as well as Keap1/Nrf2/ARE pathways and the prominent involvement of these pathways in the ARMD, the lack of CR-related studies as related to ARMD is astonishing. In Wistar rats, CR diet decreased the accumulation of lipofuscin in RPE cells [46]. Lipofuscin is an aggregate of complex material that accumulates within the lysosomes of RPE cells as a result of phagocytosis of degraded photoreceptor cells [47, 48]. The

age-related accumulation of lipofuscin may occur at an accelerated rate and cause RPE dysfunction and rapid aging of retina [47, 48]. In monkeys fed 70% CR diet, no differences in drusen were detected between CR and AL groups, but the authors suggest that the sample size in this study was too small to conclude definitively [49]. In albino Fischer 344 rats, the deleterious effect of bright light was more pronounced in rats fed CR than in AL diet [50]. However, in Brown Norway rats, CR diet exhibited a neuroprotective effects in the aging retina [2], reduced age-related photoreceptor cell death, and the reduced decline of thiols, glutathione, ascorbic acid, and taurine in the retina [51]. Although not specifically substantiated by these limited number of available studies, CR appears to possess a neuroprotective effect in retina and a potential activation of protective Keap1/Nrf2/ARE pathway in ARMD.

Benefits of CR in Glaucoma

Increased intraocular pressure, caused by defects in the ocular drainage system, is the main risk factor for glaucoma [52-54]. Increased intraocular pressure can lead to the optic nerve damage and a subsequent loss of vision and blindness. In addition, age, diabetes, and genetic factors strongly influence the occurrence of glaucoma [55]. The most common form of glaucoma is a slowly developing, chronic form of primary open-angle glaucoma (POAG). In an animal study on POAG, when albino Fischer rats, albino Wistar rats, and Brown Norway rats were fed three days a week in a CR regimen, CR protected against the loss of retinal ganglion cells (RGCs) in both young and old animals and in both albino and pigmented rats [56]. CR diet also attenuated the loss of age-related RGCs in both albino BALB/cBy mice and albino Fischer rats [57], and resisted age-related morphological alterations in aqueous collecting channel in terms of the loss of lumen area of channel and loss of anterior-posterior width [58]. Further, in albino Fischer 344 rats, CR diet attenuated age-related as well as ischemia-induced RGC loss [59]. These studies strongly suggest CR has protective effects against the loss of RGCs irrespective of the pigment color of the animal. Because RGCs express SIRT1 and SIRT1 activation provides protection against optic neuritis primarily by protecting RGCs [60], it is possible that CR-induced upregulation of SIRT1 in RGCs can also provide protection against glaucoma.

CALORIE RESTRICTION MIMETICS

CR effects have been experimentally tested on a limited number of eye diseases such as, cataract, ARMD, and glaucoma, as discussed in the previous sections. Interestingly, CRMs seem to have been tested on a broader group of eye diseases including both age-related eye diseases and eye diseases that affect all ages. Although potentially many phytochemicals may qualify to serve as CRMs, resveratrol, sulforaphane, and curcumin appear to have been tested most commonly in the eye literature, so we will discuss these three herbals along with other compelling CRMs as related to the eye disease.

Benefits of Resveratrol in Eye Diseases

There is mounting evidence in the literature suggesting the beneficial effects of resveratrol consumption and red wine drinking. Despite the fat-rich diets consumed by the French, an epidemiological finding, popularly known as "The French Paradox" has associated regular red wine consumption with a low incidence of cardiovascular disease [61]. A low incidence of vision loss among the elderly has also been linked to red wine drinking [27, 62]. Red wine and its biological source—the purple grape (*Vitis vinifera*), especially its skin and seeds—contain many polyphenols, such as flavonoids (quercetin, catechins, gallocatechin, procyanidin, prodelphidins), and resveratrol, a phytoalexin that is naturally synthesized in response to fungal attack [63-65]. In addition to its anti-aging properties, resveratrol is known to play a neuroprotective role in Huntington's disease [66], axotomy [67], Alzheimer's disease [68], brain ischemia [69], stroke [70] and epilepsy [71] by protecting brain cells from death. Recent studies also directly link the beneficial effects of resveratrol and SIRT1 activators with prevention of vision loss [27, 62, 72, 73] and activation of SIRT1 in the eye or eye cells [60, 74].

Resveratrol prevented sodium selenite-induced oxidative stress and cataract formation in an experimental cataract model in SD rats [73]. Acting as an antioxidant, resveratrol not only reduced the oxidative stress of the retinal pigment epithelium (RPE) cells, but also attenuated hyperproliferation of human RPE cells used as an *in vitro* model for ARMD [75]. In aging pigment epithelial cells, resveratrol quenched singlet oxygen and reduced A2E epoxidation, reducing the incidence of DNA damage and cell death [72]. Further, in human RPE cells, the expression of SIRT1 attenuated FOXO3 recruitment to the complement factor H (CFH) regulatory region and reversed the H_2O_2-induced repression of CFH gene expression [76]. In optic neuritis and multiple sclerosis patients, axonal damage causes vision loss and neuronal dysfunction. Although the inflammation was not suppressed, modified resveratrol formulations (SRT647 and SRT501) prevented RGC loss and protected mice from neuronal damage in experimental autoimmune encephalomyelitis (EAE), an animal model for optic neuritis and multiple sclerosis [60]. In a 34-yr old man suffering from optic neuritis, administration of red wine (0.3 dl) improved the visual acuity within 30 minutes of drinking wine, and provided temporary improvement in peripheral blood flow and visual function [77]. Among other symptoms, diabetic retinopathy is characterized by impaired ocular circulation and angiogenesis. In porcine retinal arterioles, an *in vitro* model for diabetic retinopathy, resveratrol (1-50 μM) induced the endothelium-dependent dilation or relaxation of arterioles, and this dilation was mediated by the released nitric oxide (NO) *via* NO synthase (NOS) activation by the extracellular signal-regulated (ERK) pathway, and the subsequent activation of soluble guanylyl cyclase [78]. In a pressure-induced cultured retinal ganglion cells (RGC-5), an *in vitro* model for glaucoma, resveratrol (20-40 μM) provided protection against increased oxidative stress and 4-hydroxy-2-nonenal (HNE) adducts and in C57LB/6 mice, the increased intraocular pressure (10-60 Hg) dose-dependently increased the formation of HNE and the expression of protective protein heme oxygenase-1 (HO-1) in the mice retina [79]. The trabecular meshwork (TM) cells control the outflow of aqueous humor and thus intraocular pressure in glaucoma. In pig TM cells exposed to oxidative stress (40% oxygen), resveratrol (25 μM) treatment effectively prevented increased production of intracellular reactive oxygen species (iROS) and in-

flammatory markers (interleukin (IL)1a, IL6, IL8; and endothelial leukocyte adhesion molecule 1, ELAM-1), and reduced the expression of the senescence markers sa-b-gal, lipofuscin, and the accumulation of carbonylated proteins [80]. In addition, resveratrol has exhibited anti-angiogenic effects in the eye. In C57B16/J mice implanted with vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2), resveratrol inhibited corneal neovascularization [81], and in mice injected with tumor cells that cause uveal melanoma, resveratrol (0.5-20 µM) inhibited both tumor size and volume through induction of mitochondria-mediated and caspases-3 & -9 mediated apoptosis the tumor cells [82]. Furthermore, resveratrol (50-100 µM) suppressed the cell proliferation and increased mitochondria-mediated apoptosis in Y79 retinoblastoma cells [83]. In our recent study with E1A.NR3 retinal cells, Bax was upregulated in response to the antibody treatment, but resveratrol-induced SIRT1 activated Ku70 expression in the cytoplasm and this Ku70 appear to have suppressed the movement of Bax from cytoplasm to mitochondria and prevented antibody-induced apoptosis [74]. These studies clearly suggest broad therapeutic roles of resveratrol in the eye. Resveratrol seem to possess anti-oxidant, anti-apoptotic, anti-inflammatory, anti-angiogenic, and an ability to induce both anti-aging sirtuin and Keap1/Nrf2/ARE pathways.

The previous studies have shown that CR and CRMs upregulate yeast sirtuin protein, Sir2, a nicotinamide adenine dinucleotide (NAD)-dependent class III histone deacetylase. Besides influencing many other functions, yeast Sir2 extends the animal lifespan [25, 84, 85]. In nematodes and fruit flies, Sir2 also mediates the nutrient-sensing pathway of aging [66, 86]. In rodents and human there are seven Sir2 homologues (SIRT1-7) that have been identified [87-90] and they express ubiquitously across different types of tissues [87, 88, 91]. The recent literature on mammalian SIRT1, the most studied sirtuin protein and generally implicated in longevity, suggests that its expression is highly dependent on the nature of stimuli and the type of tissue [92]. SIRT1 is generally upregulated in diseases/stress states such as, cancer, inflammation, neurodegeneration, DNA damage, oxidative stress, senescence, and CR, perhaps as a protective response [93-101], but may be downregulated during aging [92]. Under normal conditions, transcription factor p53 binds to two specific promoter sites of SIRT1 gene and block SIRT1 transcription, under mild stress, forkhead box O3a (FOXO3a) transcription factor associates with p53 and prevents its binding to the promoter site and activates the SIRT1 function such that it deacetylates E2F1, another transcription factor that binds to the SIRT1 promoter site and promotes SIRT1 transcription [92].

There are two isomeric forms of resveratrol: biologically inactive *cis*-resveratrol and active *trans*-RES (3,5,4'-trihydroxystilbene). *Trans*-resveratrol (or simply resveratrol) exhibits anti-aging effects by inducing SIRT1 protein in organisms ranging from yeasts to mammals [5, 6, 68, 102]. Although the beneficial effects of resveratrol to the eye are rapidly emerging in the literature, SIRT1 function in the eye has not been widely investigated. Because SIRT1-deficient mice have exhibited defects in the multiple layers of neuronal retina [103], SIRT1 protein possesses crucial but yet to be defined functions in the eye. Interestingly, in SirT1$^{-/-}$ ES, 293T cells, SIRT1 did not affect p53-mediated apoptosis

[104], and in Drosophila eye, overexpression of Sir2 promotes caspase-dependent but p53-independent apoptosis that is mediated by the Jun kinase (JNK) and FOXO signaling pathways [105]. In rat undifferentiated E1A-NR3 retinal cells, resveratrol-induced SIRT1 expression increased the activation of Ku70 and reduced the production of Bax and prevented antibody induced retinal cell death [74]. A polymorphism in complement factor H (CFH) may increase the risk for ARMD [76]. In human ARPE-19 cells, the overexpression of SIRT1 attenuated FOXO3 recruitment to the CFH regulatory region and reversed the H_2O_2-induced repression of CFH gene expression [76]. In C33A retinoblastoma (*Rb*-null) cells, the overexpression of SIRT1 deacetylated and inactivated Rb protein, where as inactivation of SIRT1 by siRNA increased the accumulation of Rb protein [106]. These studies strongly suggest that SIRT1 protein is likely to affect many critical ocular functions that are yet to be substantiated.

Benefits of Sulforaphane in Eye Disease

Many cruciferous vegetables such as, broccoli sprouts, cabbage, cauliflower, mustard, radish, and turnip contain a class of naturally occurring phytochemicals called isothiocyanates [107]. One such chemical is glucosinolate glucoraphanin, a precursor to sulforaphane [108, 109]. On fungal attack to the plant or injury by chewing, glucosinolate glucoraphanin is converted into active sulforaphane by an enzymatic activity involving myrosinase [107, 110]. Suforaphane is known to provide protection against many types of cancers predominantly by suppressing phase 1 enzymes such as cytochrome P450 and activating phase 2 enzymes [110, 111]. Oxidants and electrophiles produced intrinsically or from extrinsic sources function as toxicants and accelerate not only the aging process but also cause numerous age-related pathologies. Phase 2 enzymes produced within the body detoxify these toxicants and protect the organism from injury. Examples of prominent phase 2 enzymes include glutathione transferases (GSTs), glutathione reductase (GSR), glutathione peroxidase (GPX), UDP-glucuronosyltransferases (UGTs), and NAD(P)H:quinone oxidoreductase 1 (NQO1), thioredoxin reductase (TrxR), and heme oxygenase 1 (HO-1) [26]. These phase 2 genes are activated by Keap1/Nrf2/ARE pathway [26]. Under normal conditions, Keap1 binds to the transcription factor Nrf2, retains it in the cytoplasm and promotes its protesomal degradation. However, in the presence of stress-inducers, Keap1 becomes highly reactive and changes its conformation leading to the release of Nrf2. The free Nrf2 moves from cytoplasm to nucleus, where upon interacting with small Maf (sMaf) transcription factor binds to ARE and promote transcription of cytoprotective phase 2 genes [110].

In human ARPE-19 cells, an *in vitro* model for ARMD, sulforaphane (0.62-5 µM) provided protection against the ROS toxicity induced by oxidative stressors (menadione, *tert*-butyl hydroperoxide, 4-hydroxynonenal, and peroxynitrite) by upregulating phase 2 proteins such as, glutathione (GSH) and quinone reductase (QR) [112]. Similarly, sulforaphane or a bis-2-hydroxybenzylideneacetone (2-HBA) pre-treatments provided protection against retinaldehyde photosensitized oxidation of ARPE cells and fibroblast cell lines from 13.5-day-old embryos of several double-knockout mice, by inducing NQO1 and GSH [113, 114]. In these cells,

the PI3K/Akt pathway played an important role in regulating Nrf2-dependent activation of ARE and protection against oxidants [115]. Not just sulforaphane, other phytochemicals such as, fisetin, quercetin, myricetin, eriodictyol, taxifolix, epicatechin, epigallocatechin-3-gallate, and zinc metal induced the expression of Nrf2, GSH, NQO1, HO-1, glutamate-cysteine lygase (GCL) [116-118]. In male BALB/c mice, both oral and i.p. administered sulforaphane (0.1 -0.5 mg) induced thioredoxin (Trx) in mice retina and protected the retina against light-induced damage and in human K-1034 RPE cells, sulforahane induced the binding of Nrf2, small Maf, and c-Jun to the ARE of the Trx gene [119]. Further, similarly in bright-cyclic-light-reared albino Sprague-Dawley rats, sulforaphane treatment increased upregulation of the retinal levels of Trx, thioredoxin reductase (TrxR), and proteins modified by 4-hydroxynonenal (4-HNE), in addition to increased nuclear translocation of Nrf2 and the DNA binding activity of Nrf2, small Maf, and cJun to the ARE [120]. In the same study, in 661W photoreceptor cells, pretreatment with a sub-lethal dose of 4-HNE protected cells against H_2O_2-induced cell damage, by upregulating cellular Trx, TrxR, and HO-1 levels in addition to DNA binding activity of Nrf2, small Maf, and cJun to the ARE [120]. Both in Tubby mice & C57BL/6J mice, sulforaphane-induced upregulation of Trx, TrxR, Nrf2 was associated with increased activation of ERKs in preventing photoreceptor degeneration [121]. In addition, in 661W photoreceptor cells, sulforaphane and minocycline inhibited light-induced photoreceptor apoptosis partly by downregulating nuclear factor-kappaB (NF-κB) p65 subunit, but not effecting mitogen-activated protein kinase (MAPK) [122]. Similarly, in retinal microglial cells derived from Sprague-Dawley rats, these compounds inhibited lipopolysaccharide-induced retinal microglial activation by suppressing the production of inducible nitric oxide synthase (iNOS) and IL-10 [123]. These studies provide compelling evidence on the protective role of Keap1/Nrf2/ARE pathway and its cross-talk with different types of kinases in eye disease.

Benefits of Curcumin in Eye Disease

Curcumin is the main bioactive compound derived from the rhizomes (thick, modified roots) of the turmeric plant. It has been traditionally used for centuries in Indian cooking and in Ayurvedic medicine for the treatment of most debilitating diseases. In the last couple of decades, curcumin is one of the most intensively studied herbal drugs across all major divisions of medicine. This renewed scientific interest is frequently attributed to its numerous health benefits in several pathologies including eye diseases. The current eye literature seems to suggest that curcumin is most beneficial in the eye as an anti-inflammatory drug.

Curcumin at 0.002% (but not 0.01%) delayed the onset and maturation of galactose-induced cataract in Sprague-Dawley rats by exerting antioxidant and antiglycating effects, as it inhibited lipid peroxidation, AGE-fluorescence, and protein aggregation [124]. In Wistar NIN rats, curcumin (0.002-0.01%) and turmeric (0.5%) delayed the progression and maturation of STZ-induced diabetic cataract in a dose-dependent manner by reversing the hyperglycemia-induced oxidative stress, with turmeric being more effective than the corresponding dosage level of curcumin [125]. A pretreatment (24 h) with curcumin (15 µM) protected cells

against N-methyl-D-aspartic acid (NMDA)-induced excitotoxicity by reducing the intracellular calcium rise in primary retinal cell culture from Wistar rats [126]. Further, in streptotocin (STZ)-induced diabetic Lewis rat retina, curcumin (0.05%) restored antioxidant capacity, reduced oxidatively modified DNA (8-OHdG) and nitrotyrosine levels, and provided partial beneficial effects on GSH, in addition to reducing the diabetes-induced elevation of inflammatory factors IL-1β, VEGF and NF-κB [127]. In 661W and ARPE-19 cells, *in vitro* models for AMD, curcumin protected cells from H_2O_2-induced cell death and upregulated protective enzymes, such as HO-1, Trx1, and Nrf2 [128]. These studies clearly suggest that in age-related eye diseases, curcumin appears to trigger Keap1/Nrf2/ARE pathway and protect eye by upregulation of cytoprotective phase 2 genes.

Uveitis is the most prominent inflammatory response, occurring in the middle, pigmented layer of the eye called uvea (iris, ciliary body, and choroid) as well as in adjacent tissues including retina. The animals models for uveitis suggest that the process of blood cell leakage is a function of complex, well-orchestrated molecular interactions involving two critical early events: (1) activated blood cells—lymphocytes (CD4+ T helper cell subtypes Th1 and Th17 cells), monocytes (macrophages), and granular leukocytes (neutrophils), and (2) inflamed endothelial cells in the blood vessels. Activated Th1 cells secrete inflammatory cytokines such as, interferon-gamma (IFN-γ), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). Besides secreting TNF-α and IL-6, Th17 cells also secrete IL-17, a cytokine that participates in the pathology of uveitis. The secreted cytokines trigger the production of cell adhesion molecules by endothelial and other surrounding cells. In a single published article on the efficacy of curcumin in the management of chronic anterior uveitis, a group of patients who received oral administration of 375 mg of curcumin 3 times a day for 12 weeks, showed efficacy and recurrence rate comparable to corticosteroid therapy but without any adverse drug effects [129]. Topical application of aqueous solution of turmeric (0.1%) to rabbit eyes (3 times/day for 3 days) prior to the induction of LPS endotoxin-induced uveitis (EIU) significantly reduced the clinical symptoms of EIU [130]. Curcumin (10-30 µM) inhibited stroma-derived factor-1α (SDF-1α)-induced CXCR4 cytokine receptor signaling in human retinal endothelial cells (HRECs) and inhibited the growth of new blood vessels in proliferative diabetic retinopathy [131]. In addition, it dose-dependently reduced the viability of HRECs and at 10 µM concentration inhibited high glucose-induced proliferation of HRECs by attenuating VEGF-induced signaling [132]. In a recent study, the inflammatory cytokine TNF-α disrupted the barrier function by causing disappearance of zonula occluden-1 (ZO-1) protein from tight junctions and this disruption was prevented by curcumin (5-10 µM) through blocking of NF-kB protein [133]. Curcumin (20-100 µM) also dose-dependently downregulated IL-8 and monocyte chemotactic protein 1 (MCP-1) expressions in human retinal pigment epithelial cells following stimulation with glycated human serum albumin, a cell culture model for studying diabetic retinopathy [134]. These studies strongly suggest that curcumin possesses therapeutic, anti-inflammatory functions and modulates numerous inflammatory pathologies in human eye and elsewhere in the body.

BIOAVAILABILITY AND SAFETY CONCERNS RELATED TO CR MIMETICS

The bioavailability and safety of a given CRM depends on the following three considerations: (1) absorption by human colon, (2) crossing the blood-retinal barrier, and (3) causing minimal adverse drug effects [135]. Although bioavailability of none of the three prominent CRMs (resveratrol, sulforaphane, curcumin) specifically in the eye is known, based on their studies in other organs (plasma, intestine, brain), they seem to be as readily bioavailable to the eye as readily as in the brain. Some limited bioavailability data on these three CRMs are discussed below.

Resveratrol

A broad range of resveratrol doses seem to affect beneficial biological functions in the published *in vivo* bioavailability studies involving rodents and humans [63, 136-142]. The maximum tolerated dose of resveratrol in mice is 4 g/day/kg body weight for 28 days [143]. In our preliminary studies with Lewis and Sprague-Dawley rats, 100 mg/kg body weight was well tolerated by the animals, as the animals showed no signs of any adverse drug effects. Bioavailability studies in resveratrol have consistently shown that it forms glucuronide and sulfate conjugates within 30 min of injection, suggesting a very low bioavailability of original aglycone [137, 141, 142, 144]. Red wine and cooked peanuts seem to possess larger quantities of resveratrol rather than white wine and raw peanuts [136]. However, experts believe that many of the useful effects of resveratrol come not only from aglycone but also from the modified resveratrol glucuronide and resveratrol-3-sulfate conjugates that circulate in the blood stream in minute quantities.

Sulforaphane

Relative to resveratrol and curcumin only a limited number of bioavailability studies have been performed for sulforaphane. When rats were injected intravenously with sulforaphane or fed orally, sulforaphane was absorbed rapidly and showed an absolute bioavailability of 82% in the plasma and showed a dose-dependent pharmacokinetics [145]. A recent study in humans determined the bioavailability of sulforaphane after the consumption of raw and cooked broccoli [146]. Higher levels of sulforaphane conjugates were detected in blood and sulforaphane-derived mercapturic acid in urine when broccoli was consumed raw than cooked. The consumption of raw broccoli resulted in faster absorption as well as higher bioavailability (37% for raw versus 3.4% for cooked) of sulforaphane in the blood [146]. Another bioavailability study involving humans also suggested that bioavailability of isothiocyanates was three times greater in raw broccoli than the cooked broccoli [147].

Curcumin

A combination of curcumin's broad biological functions, its unique ability to be used as a dietary supplement, apparent lack of toxic effects even at extraordinary quantities, and its cheap availability in the market, make it suitable for the development of most essential preventative drugs for the treatment of high-risk patients. Federal Food and Drug Administration listed curcumin as "generally regarded as safe" drug. Although curcumin is a safe drug, it exhibits poor bioavailability after oral consumption. In a Phase I clinical trial of curcumin with 25 patients suffering from high-risk or pre-malignant lesions, the curcumin dosages were gradually increased from 0.5 g/day to 1, 2, 4, 6, 8, 12 mg/day [148]. The peak serum concentrations after 1-2 hours of oral consumption of 4, 8, and 6 g/day were 0.51 ± 0.11, 0.63 ± 0.06, and 1.77 ± 1.87 µM, respectively [148]. Although there were no toxic effects at 12 g/day dose, bulkiness of the drug was not relished. Since then numerous bioavailability studies have been conducted and a general story is that curcumin can easily reach distant organs in the body including liver, lungs, heart, and brain and affect biological functions in minute concentrations. Overall there is a limited oral bioavailability. When administered i.p. or i.v., bioavailability slightly increases but not substantially. The major reasons for the low bioavailability include: rapid metabolism, rapid systemic elimination, and poor absorption by the target tissues [149, 150]. Perhaps its poor bioavailability and yellow color appear to be the main limitations for therapeutics and drug development. Yet, curcumin is considered as the lead molecule for dozens of drugs under development. In the academia and pharmaceutical world alike, there seems to be a race towards developing curcumin formulations that enhance its bioavailability in the target tissue. Many curcumin formulations have been suggested: mixing curcumin with adjuvant piperine that blocks curcumin metabolism, or formulations with nanoparticles, liposomes, micelles, and phospholipid complexes [149, 150-152]. An optimized lipid formulation of curcumin, for example, increased its oral bioavailability by 11-fold in plasma and 4-fold in the brain compared to curcumin alone formulation (Verdure Sciences, Noblesville, Indiana). Two weeks after administration of lipidated form of curcumin in chow at 500 ppm (or 25 mg/kg body weight), the drug concentration in the mouse brain was 5.79 ± 1.22 µM, which is remarkably well above the 1-2 µM range of EC_{50} required for inhibition of iNOS, IL-1β, and PGE_2 in Alzheimer's animal models for neuroinflammation [153]. Collective evidence on curcumin bioavailability and molecular functions suggests that concentrations ranging from 10 nM to 50 µM ranges are suitable for our *in vitro* studies with human retinal endothelial cells, and wide ranging doses at milligram levels to even a gram (say 10 mg to 1 g) can be safely employed for *in vivo* studies depending on the formulations.

CONCLUSIONS

The CRM resveratrol appears to function as a pleiotropic compound that triggers multiple beneficial effects including the activation of both anti-aging sirtuin and Keap1/Nrf2/ARE pathways in age-related and eye diseases that occur in all ages. Both sulforaphane and curcumin also exhibit pleiotropic beneficial effects that include the Keap1/Nrf2/ARE pathway, but not the sirtuin pathway. While sulforaphane seem to exert its beneficial functions in age-related diseases, curcumin appears to affect beneficial functions in both age-related and diseases such as uveitis that may occur at any age.

ACKNOWLEDGEMENTS

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