Induction of the Glutathione Antioxidant Response/Glutathione Redox Cycling by Nutraceuticals: Mechanism of Protection against Oxidant-Induced Cell Death

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Abstract

The “Mitochondrial Free Radical Theory of Aging” (MFRTA) hypothesizes that reactive oxygen species (ROS) arising from aged and/or defective mitochondria are associated with the pathogenesis of various age-related diseases. The glutathione antioxidant response, in particular glutathione redox cycling, is a critical mechanism for protection against ROS-induced cell death. Over the past few decades, a number of phytochemicals [such as curcumin, epigallocatechin gallate (EGCG), resveratrol and schisandrin B (Sch B)], which all possess the ability to elicit a nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-mediated antioxidant response, have been identified. Despite the fact that these phytochemicals can produce cyto/tissue protection against oxidant-induced injury in various types of cultured cells/rodent tissues, the underlying protective mechanism can vary. While curcumin, EGCG and resveratrol likely confer cytoprotection via the activation of glutathione S-transferase and glutathione peroxidase, Sch B is thought to produce its protective effect via the induction of glutathione redox cycling, which is of primary importance in preventing cell death. Recent studies have suggested that the electrophilicity of phytochemicals and/or their metabolites determines their ability to activate Nrf2 by the oxidative modification of a cysteine residue on the repressor of Nrf2 [namely, Kelch-Like ECH-Associated Protein 1 (Keap1)]. The differences in structures of phytochemicals could produce differential accessibility to this critical cysteine residue of Nrf2/Keap1, presumably leading to varying degrees of Nrf2 activation and antioxidant gene expression. In the hope of developing safe and effective interventions for protection against oxidant-induced injuries, further studies are required to define the protective mechanism(s), particularly the array of antioxidant enzyme expressions, induced by the various phytochemicals.

Keywords: Glutathione; Glutathione redox cycling; Resveratrol; Schisandrin B; Epigallocatechin gallate; Curcumin

Abbreviations

EGCG: Epigallocatechin Gallate; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; Grx: Glutaredoxin; GSH: Reduced Glutathione; GSSG: Oxidized Glutathione; GST: Glutathione S-transferase; ICDH2: Isocitrate Dehydrogenase II; Keap1: Kelch-like ECH-Associated protein 1; MFRTA: Mitochondrial Free Radical Theory of Aging; mtDNA: mitochondrial DNA; Nrf2: Nuclear Factor (erythroid-derived 2)-like 2; PSSG: mixed disulfide Protein; ROS: Reactive Oxygen Species; Sch B: Schisandrin B; γ-GCL: γ-Glutamyl Cysteine Ligase

Oxidative Stress and Oxidative Stress-related Diseases

The “Mitochondrial Free Radical Theory of Aging” (MFRTA) hypothesizes that the gradual and continuous production of reactive oxygen species (ROS) in mitochondria can cause oxidative lesions in mitochondrial DNA (mtDNA), with resultant mitochondrial dysfunction [1]. Defects in mitochondria can further potentiate the generation of ROS, leading to catastrophic and irreversible damage to the cell. Over the past few decades, oxidative stress has been found to be associated with the pathogenesis of a number of diseases, e.g. cancers, metabolic disorders and neurodegenerative diseases [1-3]. In this regard, mitochondrial ROS were found to activate a hypoxia-inducible factor as well as phosphoinositide 3-kinase pathways and induce metabolic changes, which in combination favor cell survival, growth and proliferation, with a resultant increased risk of carcinogenesis [2]. In addition, mitochondrial defects arising from germline mtDNA variations and somatic mtDNA mutations are predisposing factors for the pathogenesis of neurodegenerative diseases [3]. Obesity, which is a predisposing factor for metabolic diseases, was also found to be correlated with increased ROS generation and/or the inadequacy of endogenous antioxidant defense systems [4]. A recent review updating the MFRTA has suggested that a low generation rate of endogenous damage and the presence of macromolecules with high resistance to oxidative modification in mitochondria are likely to increase the longevity of organisms [5]. It therefore follows that the
capability of aerobic organisms to cope with unavoidable oxidative challenge is of fundamental importance.

Components and Physiological Functions of the Glutathione Antioxidant System

Glutathione, a tripeptide containing glutamic acid, cysteine and glycine, accounts for approximately 90% of non-protein low molecular weight thiols in mammalian tissues [6]. Glutathione can exist in two redox isoforms - namely, the thiol-reduced form (GSH) and the disulfide-oxidized (GSSG) form. Instead of being linked by a conventional dipeptide bond, glutamate and cysteine residues are linked by a reaction catalyzed by γ-glutamyl cysteine ligase (γ-GCL), in which a covalent bond is formed between the γ-carboxyl group of glutamine and the amino group of cysteine, with a resultant resistance to intracellular degradation by proteases. As a primary antioxidant in the cell, glutathione actively participates in antioxidant defense processes in various ways. Firstly, the free radical scavenging activity of GSH is attributable to the thiol group of its cysteine residue [7].

Secondly, the higher reducing potential of GSH also enables the reduction of dehydroascorbate (the oxidized form of ascorbate) to ascorbate [8], which in turn regenerates the phenoxyl radical of α-tocopherol back to α-tocopherol in cell membranes [9]. The GSH-coupled redox reactions ensure the efficient regulation of cellular antioxidant capacity via the maintenance of an optimal GSH/GSSG ratio. Finally, GSH serves as a co-substrate of several antioxidant enzymes – notably, glutathione S-transferase (GST) and selenium-glutathione peroxidase (GPx). While GPx catalyzes the reduction of both organic and inorganic hydroperoxides into their corresponding alcohols at the expense of GSH, GST inactivates electrophiles by catalyzing the transfer of a glutathiolate group to their reactive molecular moieties.

To maintain the high efficiency of both non-enzymatic and enzymatic antioxidant networks, the availability of GSH is essential. GSSG can be regenerated by reduction to GSH by glutathione reductase (GR) at the expense of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is produced in the pentose phosphate pathway [in which glucose-6-phosphate dehydrogenase (G6PDH) is the enzyme catalyzing the rate-limiting step] or isocitrate dehydrogenase II (ICDH2)-catalyzed reactions. Glutathione and glutathione-related enzymes, which are collectively referred to as the "glutathione antioxidant system", provide a generalized protection against oxidative stress in cytosolic and mitochondrial compartments (Figure 1).

Regulation/Induction of the Glutathione Antioxidant System

Regulation/Induction of the Glutathione Antioxidant System

Despite the fact that an excessive production of ROS is deleterious, accumulating evidence suggests an important physiological role of ROS as signal transduction molecules [10]. The redox-sensitive nuclear factor (erythroid-derived 2)-like 2 (Nrf2)/antioxidant response element pathway, which induces a cytoprotective response against oxidative injury, is primarily activated by pro-oxidant or oxidants. Under normal (i.e., non-stressful) conditions, Nrf2, which is bound by its specific repressor, namely, Kelch-like ECH-associated protein 1 (Keap 1), in the cytosol, is subjected to ubiquitination, with a resultant ubiquitin-mediated proteasomal-catalyzed degradation, resulting in inactivation [11,12]. As such, the dissociation of Nrf2 and Keap1 can promote the nuclear translocation of Nrf2 and the subsequent expression of antioxidant genes. In this respect, it has been shown that the translocation of Nrf2 from the cytosol to the nucleus is facilitated by phosphorylation of Nrf2 [13] or oxidative modification of the cysteine residue of Keap1, which can dissociate Nrf2 from Keap1 [14]. This leads to a subsequent enhancement in the expression of antioxidant defense genes, including the catalytic and modulatory subunit of γ-GCL, GR, GPx and GST, which encompass the glutathione antioxidant response [15].

Regulation of Apoptosis by the Glutathione Antioxidant System

Mitochondrial glutathione redox status (i.e. GSH/GSSG ratio) is the determining factor for the activation of apoptosis, as evidenced by a study showing that a transient and rapid oxidation of GSH [i.e. a decrease in the ratio of GSH to GSSG] is sufficient to trigger a mitochondrial apoptotic pathway, which cannot be reversed by a subsequent restoration of glutathione redox status [16]. The depletion of cellular GSH can lead to apoptosis, presumably via the activation of mitogen-activated protein kinase cascades, as demonstrated in various cell models [17,18]. In addition, the depletion of mitochondrial GSH was found to be associated with a range of apoptotic stimuli (such as exposures to hypoxia, oxidants and...
Components of the glutathione antioxidant system are actively involved in the regulation of oxidant-induced apoptosis. In addition to the decomposition of organic hydroperoxides, GST catalyzes the transfer of a glutathiolate group to electrophiles, which can otherwise covalently modify biomolecules such as proteins, lipids and DNA. This reversible S-glutathionylation on cysteine residues in biomolecules is hypothesized to be a mechanism protecting against the irreversible oxidative modification of biomolecules [23]. The reversal of S-glutathionylation (i.e., de-glutathionylation) is catalyzed by glutaredoxin (Grx) at the expense of GSH as a co-substrate [24].

Recently, the complex regulatory role of S-glutathionylation and de-glutathionylation in apoptosis has been elucidated. Firstly, under conditions of oxidative stress, the protein sulfenic acid (PSOH, a detrimental oxidative modification of a protein thiol) can further undergo GST-catalyzed S-glutathionylation to form a mixed disulfide protein (PSSG) that does not elicit apoptosis [25]. Secondly, the S-glutathionylation of the “death receptor” (namely, Fas) was found to increase the binding efficiency of Fas for its receptor, leading to apoptosis [26]. Thirdly, the up-regulation of Grx can result in the reduction of PSSG to PSH, resulting in protection against cytochrome c-mediated apoptosis [27]. Finally, the de-glutathionylation of caspase 3 was also found to promote apoptosis [28].

In summary, the reservoirs of GSH in mitochondria and the cytosol serve as critical determinants in maintaining an optimal glutathione redox status as well as an appropriate degree of S-glutathionylation/de-glutathionylation of regulatory molecules. Cellular levels of GSH are determined by two reactions - namely, de novo GSH synthesis and GSH regeneration. The biosynthesis of GSH occurs in the cytosol of all cell types [29]. It involves two anabolic reactions which are catalyzed by 2 ATP-dependent enzymes, namely, γ-GCL and glutathione synthetase. The first step, which is catalyzed by γ-GCL, is considered to be the rate-limiting step in GSH synthesis [30]. GSH regeneration, in which GSSG is reduced into GSH at the expense of NADPH, is catalyzed by GR. Nrf2-regulated glutathione redox cycling, rather than GSH biosynthesis, was found to be crucial for cell survival during oxidative stress [31]. Therefore, the most effective way to confer protection against oxidant-induced cell injury is the enhancement of GSH recovery capacity [32], which is presumably mediated by the induction of Nrf2-regulated cytoprotective proteins such as GR and NADPH-producing enzymes via a redox signaling pathway.

Phytochemicals as Inducers of the Glutathione Antioxidant Response and their Cytoprotective Effects

In view of the increased morbidity and mortality associated with oxidative stress-related diseases, antioxidant interventions are urgently being sought. In an effort to develop safe and effective therapies for oxidant-induced injuries, the use of phytochemicals, with their ability to activate the Nrf2-mediated antioxidant response, has attracted a great deal of interest [33]. It has been suggested that electrophilic or pro-electrophilic (i.e., becomes electrophilic after metabolism) phytochemicals (which is defined as naturally occurring bioactive chemicals isolated from plants) can activate Nrf2/Keap1 via the oxidative modification of the regulatory cysteine residue(s) on Nrf2/Keap1. Pro-electrophilic phytochemicals, which are activated by enzyme-catalyzed reactions and do not cause excessive depletion of cellular GSH, are desirable candidates as Nrf2 inducers [34].

Among pro-electrophilic phytochemicals, ortho- and para-catechol (also called hydroquinone) but not meta-catechol can be oxidized into their corresponding quinones, which in turn can modify the regulatory sulfhydryl residue of cysteine in Keap1. In this review, various well-known Nrf2 inducers isolated from plants will be discussed in relation to their ability to induce a glutathione antioxidant response.

Curcumin

Curcumin is one of the principal curcuminoids obtained from the root of Curcuma longa that has been widely used in ancient Ayurvedic medicine (Figure 2).

Recent studies have demonstrated that curcumin induces an antioxidant response via the Nrf2 pathway [35-38]. The ability of curcumin to activate Nrf2 is likely attributed to its electrophilic properties (i.e. it possesses two α, β unsaturated carbonyl groups and thereby serves as a Michael reaction acceptor), and its capacity to modify critical cysteine residues.
on Keap1. In this regard, curcumin has been shown to attenuate the extent of GSH depletion via the induction of Nrf2 in various experimental settings, such as glucose oxidase-induced insulin resistance in cultured human hepatocytes [35], high fat diet-induced non-alcoholic steatohepatitis in rats [36] and arsenic-induced hepatotoxicity in mice [37]. Curcumin has also been shown to increase the activity of GST in HepG2 human hepatocytes [38]. The ability of curcumin to increase cellular GSH levels under conditions of oxidative stress was demonstrated in cultured astrocytes [39] and in isolated rat cerebellar granule neurons [40]. The neuroprotection afforded by curcumin was further demonstrated in quinolinic acid-induced neurotoxicity in rats, through the induction of Nrf2 and the activation of Gpx [41].

Epigallocatechin gallate

Epigallocatechin gallate (EGCG), which is the principal active catechin found in green tea, can induce Nrf2 and elicit an antioxidant response (figure 2) [42-44].

Structure-activity relationship studies of various catechins in the induction of Nrf2-mediated antioxidant responses have demonstrated that the pyrogallol moiety in EGCG is critical for Nrf2 activation, which involves the conversion of pyrogallol into its corresponding electrophilic quinone moiety by biotransformation [42]. This suggests that EGCG is a pro-electrophilic Nrf2 inducer. EGCG can confer hepatoprotection against oxidant injury by increasing hepatic GSH levels and Gpx activity in concanavalin A-intoxicated mice [45] and bile duct-ligated mice [46]. Cardioprotection against doxorubicin toxicity in mice [47] and neuroprotection against ischemia/reperfusion injury in rat cerebrum [44] afforded by EGCG were associated with the activation of Nrf2 and increases in γ-GCL activity and GSH levels. EGCG can induce the Nrf2-mediated expression of γ-GCL and Gpx, with resultant protection against crescentic glomerulonephritis in mice [43]. EGCG has also been shown to increase GSH levels and Gpx activity in cisplatin-induced nephrotoxicity in mice [48].

Resveratrol

Resveratrol is a stilbene present in grape skins, peanuts as well as in red wine (figure 2).

Although resveratrol is a well-known Nrf2 inducer, the underlying mechanism in relation to the molecular structure of resveratrol or its metabolites has not been clearly defined. A recent study has demonstrated that a synthetic hydroxylated analog of resveratrol, namely 3,4-dihydro-trans-stilbene, can activate Nrf2, presumably due to the presence of a catechol hydroxyl moiety in the 3,4 position that can under auto-oxidation into an ortho-quinone moiety [49]. This suggests that resveratrol is a pro-electrophilic Nrf2 inducer. Resveratrol was found to increase the Nrf2-mediated expression of Gpx and GST in isolated rat hepatocytes [50] and in diabetic rat livers [51]. Resveratrol can also protect against arsenic trioxide-induced cardiotoxicity [52] and myocardial ischemia/reperfusion injury [53] in rats, with associated increases in Nrf2 nuclear translocation, glutathione redox ratios and Gpx activity. The neuroprotection afforded by resveratrol in streptozotocin-diabetic rats [54] and in young spontaneously hypertensive rats [55] correlated well with the induction of the Nrf2-mediated expression of GST.

Schisandrin B

Schisandrin B (Sch B) is the most abundant dibenzocyclooctadiene lignan found in the fruit of Schisandra chinensis (Figure 2), a Chinese herb traditionally used for the treatment of hepatitis [56].

Recent studies have demonstrated that Sch B can elicit a glutathione antioxidant response via the ERK/Nrf2/ARE pathway [57,58]. The ability of Sch B to activate Nrf2 is likely due to the pro-electrophilic properties of Sch B, which is converted to a catechol metabolite by cytochrome P450-catalyzed dimer thlenylation of the methylenedioxy moiety [59]. The resultant ortho-catechol metabolite of Sch B can then be oxidized into a corresponding quinone that may give rise to the generation of ROS or modify Keap1, leading to the activation of Nrf2. In this regard, Sch B was found to induce a glutathione antioxidant response (including an elevation in cellular/mitochondrial GSH levels and increased expression of GR, γ-GCL and G6PDH) in rodent brain, heart and liver in vitro and in vivo, with associated neuro/cardiac/hepatic protection against oxidant-induced injuries [57-63].

Comparison among various Nrf2-inducing phytochemicals in the induction of the glutathione antioxidant response

Results concerning the ability of phytochemicals to elicit a Nrf2-mediated glutathione antioxidant response vary among studies, presumably due to variations in experimental conditions, e.g., differences in concentration(s)/dose(s) and cell types being used. In this regard, our laboratory has conducted comparative studies of the four aforementioned phytochemicals with respect to their ability to induce a glutathione antioxidant response in vitro and in vivo, using the same concentrations and/or dose [60,64]. The neuroprotective effects of curcumin, EGCG, resveratrol and Sch B in β-amyloid-intoxicated human neuroblastoma SH-SY5Y cells were investigated [60]. While curcumin and resveratrol did not activate the glutathione antioxidant response or protect neuronal cells against β-amyloid-induced apoptosis, EGCG and Sch B did afford protection, although the mechanisms differed. In this model of injury, EGCG did not activate Nrf2, GR or G6PDH, so that its attenuation of the oxidant-induced depletion of GSH was presumably due to its free radical scavenging activity. In addition to activating Nrf2 and GR, Sch B also elevated G6PDH activity, which would sustain the generation of NAPDH for the efficient GR-catalyzed regeneration of GSH from GS, with a consequent attenuation of oxidant-induced GSH depletion, resulting in neuroprotection. As the bioavailability of various phytochemicals in vivo may vary, the protective effects of long-term, low-dose oral treatment with curcumin, EGCG, resveratrol and Sch B on oxidant injury was also investigated in rat heart and liver in relation to their ability to increase glutathione recovery capacity [64]. Among the tested phytochemicals, Sch B and resveratrol (but not curcumin or EGCG) could protect the liver against carbon tetrachloride
toxicity, whereas treatment with Sch B or curcumin (but not EGCG or resveratrol) conferred cardioprotection against ischemia/reperfusion injury in rats. When the effects of phytochemicals on glutathione recovery capacity in rat heart and liver were compared, only Sch B (but not curcumin, EGCG or resveratrol) increased glutathione recovery capacity in tert-butyl hydroperoxide-challenged heart and liver homogenates. Results obtained from these studies strongly suggest a correlation between the enhancement of glutathione recovery capacity and cyto/tissue protection against oxidant injury following Sch B incubation/treatment. Other phytochemicals also protect against oxidative injury but without an enhancement of glutathione recovery capacity, possibly through other protective mechanisms. It has been reported that the protection against oxidative stress afforded by the four tested phytochemicals was associated with increases in cellular GSH level as well as GST and GPx activities [68]. However, the effects of curcumin, EGCG and resveratrol on GR and G6PDH activities were not always reported. Conceivably, curcumin, EGCG and resveratrol might confer cyto/tissue protection against oxidative stress via a GST-catalyzed S-glutathionylation of important proteins against irreversible oxidative modification, a GST-catalyzed inactivation of electrophiles or a GPx-catalyzed reduction of organic/inorganic peroxides. In addition, many cellular antioxidant thiols require a reducing equivalent (i.e. NADPH) for the enzymatic regeneration of the respective reduced form. In this regard, the ability of phytochemicals to activate the pentose phosphate pathway in the cytosol or the ICDH2-catalyzed NADH conversion in mitochondria are crucial for conferring tolerance/resistance against acute oxidative stress. Consistent with this, Sch B can increase the activities of G6PDH and ICDH2 (as well as GR) in rodent hearts and livers [unpublished data], suggesting that the cyto/tissue protection afforded by Sch B may be mainly mediated by an enhancement of the glutathione recovery capacity. An in-depth study of the activation of an array of antioxidant genes by different phytochemicals remains to be conducted.

Despite the fact that the four tested phytochemicals are well-known Nrf2 inducers, they belong to different subcategories of polyphenolics based on their chemical moieties. As mentioned earlier, curcumin is likely to act as an electrophilic Nrf2 inducer, while EGCG, resveratrol and Sch B are likely pro-electrophilic Nrf2 inducers. In this regard, Staoh et al. have suggested that pro-electrophilic phytochemicals, which act as pro-drugs that requires bio-activation, are more promising therapeutic candidates than are electrophilic phytochemicals, which act directly on cellular thiols, in order to produce an antioxidant response [34]. This is because electrophilic phytochemicals can react directly with cellular thiols (such as glutathione), and produce oxidative stress in normal cells. Despite the fact that curcumin, by virtue of its high electrophilicity, is likely to be a direct Nrf2 inducer, curcumin was found to be rapidly and efficiently metabolized into glucuronide and sulfate conjugates in intestines and then excreted in feces and urine in rats, with a resultant low oral bioavailability [65]. To reconcile the low oral bioavailability of curcumin, Kanai et al. developed a nanomized preparation of curcumin with increased water solubility, which has an improved oral bioavailability in humans [66]. Given their different molecular structures, EGCG, resveratrol and Sch B are likely metabolized by different CYP-catalyzed processes [67], with a resultant generation of stereo-specific electrophiles. The differing structures of the electrophiles might result in differential accessibility to the regulatory cysteine residue of Nrf2/Keap1, resulting in differing extents of Nrf2 activation and antioxidant gene expression. Sch B metabolites, which are generated from CYP-catalyzed reactions, have been recently identified in mouse liver microsomes in vitro and in mouse urine in vivo, using liquid chromatography coupled electrospray ionization tandem mass spectrometry (LC/MS) [59]. Apparently, ROS generated from the redox cycling of quinone metabolite is responsible for the activation of Nrf2 [68]. In addition, EGCG was found to be a pro-oxidant and undergo an auto-oxidation under physiological pH [69]. Similar to the metabolism of Sch B, the possession of a catechol hydroxyl moiety (which is readily auto-oxidized into a quinone) in EGCG can also facilitate the production of ROS via redox cycling [70], leading to the induction of antioxidant response [71]. Recently, resveratrol was found to be metabolized into piceatannol in vitro in rat liver microsomes, using electrochemical liquid chromatography as well as LC/MS [72]. Interestingly, piceatannol, which possesses a catechol hydroxyl moiety, has been reported to induce the Nrf2-mediated expression of heme oxygenase 1 in cultured mammalian epithelial cells [73]. In this regard, the signaling ROS arising from the redox cycling of quinone metabolites of various phytochemicals are likely to be primarily responsible for eliciting antioxidant response in vivo.

Conclusions

The induction of the glutathione antioxidant response, particularly the activation of glutathione redox cycling, plays an important role in protecting against oxidant-induced cell death. Despite the fact that a number of phytochemicals can elicit a Nrf2-mediated antioxidant response, the underlying protective mechanism of a given phytochemical limits its ability to protect against different types of oxidative stress and hence its potential therapeutic application. Sch B primarily induces the glutathione antioxidant response and has been shown to consistently protect against oxidant-induced cell death/injury in various in vitro and in vivo experimental systems, whereas curcumin, EGCG and resveratrol likely confer protection via other antioxidant/cellular actions. Further studies are required to define the protective mechanism, particularly the array of antioxidant enzyme expressions, induced by various phytochemicals. Hopefully, new therapeutic strategy using phytochemicals can be developed for safeguarding health and ameliorating the pathogenesis of oxidative stress-related diseases.

References


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