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REVIEWS: CURRENT TOPICS

Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes☆

Roberta Masella*, Roberta Di Benedetto, Rosaria Varì, Carmela Filesi, Claudio Giovannini

National Centre for Food Quality and Risk Assessment, Istituto Superiore di Sanità, 00161 Rome, Italy

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Abstract

Polyphenols are wide variety of compounds that occur in fruits and vegetables, wine, tea, extra virgin olive oil, chocolate and other cocoa products. Several polyphenols have been demonstrated to have clear antioxidant properties in vitro, and many of their biological actions have been attributed to their intrinsic reducing capabilities. However, this concept appears now to be a simplistic way to conceive their activity. Evidence is indeed accumulating that polyphenols might exert several other specific biological effects that are as yet poorly understood. In this article we review the most recent data on the subject and describe the additional functions that polyphenols can have in biological systems, focusing on their effects on glutathione and its related enzymes. Experimental data indicate that polyphenols may offer an indirect protection by activating endogenous defense systems. Several lines of evidence suggest a tight connection between exogenous and endogenous antioxidants that appear to act in a coordinated fashion. It is reasonable to hypothesize that this is achieved, at least in part, through antioxidant responsive elements (AREs) present in the promoter regions of many of the genes inducible by oxidative and chemical stress. The latest studies strongly suggest that dietary polyphenols can stimulate antioxidant transcription and detoxification defense systems through ARE.

Keywords: Polyphenols; Glutathione; ARE; Nrf2

1. Introduction

Oxidation reactions are an essential part of normal metabolism as oxygen is the ultimate electron acceptor in the electron flow system that produces ATP [1]. Problems may arise when electron flow and energy production become uncoupled so that oxygen free radicals, that is, reactive oxygen species (ROS), are produced [2]. Actually, ROS are continuously produced within the cell as a result of mitochondrial electron transfer processes or as bioproducts of the enzymes xantine oxidase, lipoxygenases and cycloxygenases [3]. Furthermore, ROS can be generated as a consequence of the intracellular metabolism of foreign compounds, toxins or drugs by cytochrome *P*450, monoxygenases, or because of exposure to environmental factors such as excessive iron salts or UV irradiation [4]. Other sources of ROS are macrophages and neutrophils that contain enzymes, such as NADPH oxidase complex, able to generate superoxide radicals and hydrogen peroxide [5]. Reactive oxygen species thus play different positive roles in vivo, being involved in energy production, phagocytosis, cell growth and intercellular signalling regulation. Reactive oxygen species may be also highly damaging, as they can attack biological macromolecules, namely, lipids, proteins and DNA, induce oxidation and cause membrane damage, enzyme inactivation and DNA damage [6,7]. However, when the level of ROS exceeds the antioxidant capacity of the cell, the intracellular redox homeostasis is altered and oxidative stress ensues [8]. Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative diseases, such as atherosclerosis, cardiovascular disease, type 2 diabetes and cancer [9–11]. In order to cope with an excess of free radicals produced upon oxidative stress, humans have developed sophisticated mechanisms in order to maintain redox homeostasis. These protective mechanisms either scavenge or detoxify ROS, block their production, or sequester transition metals that are the source of free

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* Corresponding author. Tel.: +39 649902763; fax: +39 649902763.
E-mail address: masellar@iss.it (R. Masella).

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radicals, and include enzymatic and nonenzymatic antioxidant defenses produced in the body, namely, endogenous [12,13], and others supplied with the diet, namely, exogenous [14–16]. Among these, natural polyphenol compounds have been largely studied for their strong antioxidant capacities and, recently, for additional properties by which cell activities are regulated. These additional properties are discussed in this article by reviewing the most recent data on the subject and focusing on the effects of polyphenols on endogenous antioxidant defenses, namely, glutathione (GSH) and related enzymes.

2. Exogenous antioxidants

Many compounds in plants and vegetables have the ability of reacting with free radicals without generating further radicals, therefore, quenching chain reactions. Other compounds scavenge ROS and in so doing they become oxidized and need to be regenerated for further use. Antioxidant compounds react directly with radicals reducing oxidative stress and exerting their protective effects against cellular damage [17–19]. Polyphenols comprise a wide variety of compounds, divided into several classes (i.e., hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanindins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes and lignans), that occur in fruits and vegetables, wine and tea, chocolate and other cocoa products [20]. Epidemiological studies showed that increased intake of polyphenols was associated with reduced risk of cardiovascular diseases, cancer and neurodegenerative disorders [21–26]. The beneficial effects of polyphenols are mainly ascribed to their capacity to counteract conditions of oxidative stress that accompany these pathologies. Several polyphenols have been demonstrated to have clear antioxidant properties in vitro as they can act as chain breakers or radicals scavengers depending on their chemical structures, which also influence their antioxidant power [27–31]. A hierarchy has been established for the different polyphenolic compounds within each class on the basis of their capability to protect lipids, proteins or DNA against oxidative injury [32–37]. As a consequence, many of their biological actions have been attributed to those antioxidant properties [38]. This concept, however, appears now to be a simplistic way to conceive their activity [39]. First of all, pro-oxidant effects of polyphenols have also been described [40] to have opposite effects on basic cell physiological processes: for example, if as antioxidants they improve cell survival, as pro-oxidants they may indeed induce apoptosis, cell death and block cell proliferation [41]. It should be noted that intracellular redox status, which is influenced by antioxidants, can regulate different factors, that is, NFκB, which in turn regulate various cell activities [42–44]. On the other hand, accumulating evidence indicates that polyphenols exhibit several additional properties in complex biological systems, but which are as yet poorly understood [45–47]. This is also suggested by at least two considerations. First of all, phenolic compounds are metabolized in vivo, giving rise to compounds that lose the original antioxidant potential, which is mainly determined by a dihydroxylated B-ring (catechol group), a 2,3-unsaturation associated with a 4-oxo function in the C-ring, and functional groups capable of chelating transition metals [48,49]. Secondly, concentrations of polyphenols and their metabolites, in vivo, in plasma or tissues, are lower than those of other antioxidants such as ascorbic acid and α-tocopherol, which renders their competition unlikely [50–53]. On the contrary, the concentrations of different polyphenols in vivo can have a pharmacological action that modulates several cell activities. Experimental data are available about the multiple potential biological activities of polyphenols: (i) inhibition or reduction of different enzymes such as telomerase [54], cycloxygenases [55–57], lipoxygenases [58,59], xanthine oxidase [60], metalloproteinase [61,62], angiotensin-converting enzyme [63], protein kinases [64,65]; (ii) interaction with signal transduction pathways [66–68]; (iii) interaction with cell receptors [69,70]. Polyphenols may also interact with caspase-dependent pathways [71–73]; interfere with cyclin-dependent regulation of the cell cycle [74]; induce detoxifying enzymes [75]; enhance the production of vasodilating factors such as nitric oxide [76,77]; affect the platelet function [78]; compete with glucose for transmembrane transport [79]. It is mainly by virtue of these properties that polyphenols exert their protective effects and receive more and more attention as therapeutic agents against cancer and cardiovascular diseases [75,80]. Experimental data indicate that they may also offer an indirect protection by activating endogenous defense systems.

3. Endogenous antioxidants

Several antioxidant enzymes exist that convert ROS into less noxious compounds, for example, superoxide dismutase (SOD), catalase, thioredoxin reductase, peroxiredoxin and glutathione peroxidase (GPx) [81–85]. Collectively, these enzymes provide a first line of defense against superoxide and hydrogen peroxides. They are of enormous importance in limiting ROS-mediated damages to biological macromolecules, but they are not able to be 100% effective because certain compounds generated by the interaction of ROS with macromolecules are highly reactive. It is then mandatory to detoxify these secondary products in order to prevent further intracellular damage, degradation of cell components and eventual cell death. This second line of defense against ROS is provided by enzymes such as GPx, glutathione *S*-transferase (GST), aldo-keto reductase and aldheyde dehydrogenase [86–88]. The detoxified metabolites produced by these enzymes are eliminated from the cell by efflux pumps such as the glutathione *S*-conjugate transporter [89]. Thus, the central role of reduced GSH appears clear in intracellular endogenous antioxidant

defenses as it is involved in all the lines of protection against ROS [13].

4. Glutathione and related enzymes

The tripeptide γ-glutamylcysteinylglycine or GSH is the major nonenzymatic regulator of intracellular redox homeostasis, ubiquitously present in all cell types at millimolar concentration [90]. This cysteine-containing tripeptide exists either in reduced (GSH) or oxidized (GSSG) form, better referred to as glutathione disulfide, and participates in redox reactions by the reversible oxidation of its active thiol [91,92]. Under normal cellular redox conditions, the major portion of this regulator is in its reduced form and is distributed in nucleus, endoplasmic reticulum and mitochondria. In addition, GSH may be covalently bound to proteins through a process called glutathionylation and acts as a coenzyme of numerous enzymes involved in cell defense [93]. Glutathione can thus directly scavenge free radicals or act as a substrate for GPx and GST during the detoxification of hydrogen peroxide, lipid hydroperoxides and electrophilic compounds. Glutathione peroxidases constitute a family of enzymes, which are capable of reducing a variety of organic and inorganic hydroperoxides to the corresponding hydroxy compounds, utilizing GSH and/or other reducing equivalents. There are several tissue-specific GPx's that exhibit also tissue-specific functions [94]. All of them are selenoproteins and their primary function is to counteract oxidative attack. During the catalytic cycle, selenium is oxidized by the hydroperoxide to a selenic acid derivative. This intermediate is subsequently reduced by the electron donor. When GSH is used, a seleno-disulfide is formed, which is cleaved by a second GSH molecule to yield the reduced GPx. During catalysis the oxidation state of the enzyme depends on the relative concentration of the reducing (GSH) and oxidized (hydroperoxides) substrates. Glutathione peroxidases are ubiquitously distributed. In the gastrointestinal tract the isoenzyme provides a barrier against hydroperoxides derived from the diet or from the metabolism of ingested xenobiotics. The phospholipid hydroperoxide GPx—discovered as a factor preventing lipid peroxidation—is considered to be involved in the protection of biomembranes against oxidative stress. In general, these isoenzymes may have a role in the regulation of the delicate regional redox balance, in particular the regulation of the appropriate tone of hydroperoxides known to be involved in cellular signaling, and to evoke several cellular responses, for example, programmed cell death, proliferation, cytokine production, and so on [95]. Glutathione *S*-transferases are three enzyme families—cytosolic, mitochondrial and microsomal—that detoxify noxious electrophilic xenobiotics, such as chemical carcinogens, environmental pollutants and antitumor agents. Moreover, they protect against reactive compounds produced in vivo during oxidative stress by inactivating endogenous unsaturated aldehydes, quinones, epoxides and hydroperoxides, all of which are produced intracellularly after the exposure to pollutants, or consumption of overcooked or mycotoxin-contaminated food, or polluted water [96]. Glutathione *S*-transferases exert those protective effects because they are able to catalyze the conjugation of GSH with oxidation end products and represent a second line of defense against the highly toxic spectrum of substances produced by ROS-mediated reaction. Both GPx and GST activities can eventually lower the level of total intracellular GSH. During the course of the reaction catalyzed by GPx, the exaggerated production of GSSG can lead to the formation of mixed disulfides in cellular proteins, or to the release of GSSG excess by the cell, to maintain the intracellular GSH/GSSG ratio. During the GST-mediated reactions, GSH is conjugated with various electrophiles and the GSH adducts are actively secreted by the cell. Mixed disulfide formation together with GSSG or GS-conjugated efflux can result in the depletion of cellular GSH, which can be opposed by a de novo synthesis or by reducing the formed GSSG. Glutathione is synthesized in two sequential ATP-dependent reactions catalyzed by γ-glutamylcysteine synthetase (γ-GCS)—the rate-limiting enzyme—and glutathione synthetase. Other factors in the regulation of the de novo GSH synthesis are the availability of cysteine and the concentration of GSH itself that inhibits, by a feedback mechanism, γ-GCS activity [97]. In the presence of oxidative stress, GSH concentration rapidly decreases while GSSG—potentially highly cytotoxic—increases because of the reduction of peroxides or as a result of free radical scavenging. This has two important consequences: (1) the thiol redox status of the cell will shift and activate certain oxidant response transcriptional elements, and (2) GSSG may be preferentially secreted from the cell and degraded extracellularly, increasing the cellular requirement for de novo GSH synthesis. Glutathione disulfide can also be reduced back to GSH by the action of glutathione reductase (GRed) utilizing NADPH as a reductant [98]. Glutathione reductase is a flavoenzyme and is represented by a single-copy gene in humans. It has been observed that exposure to agents that lead to increased oxidative stress also leads to an increase in its mRNA content. Further experimental data have shown the importance of GRed activity in GSH metabolism, demonstrating that the enzymatic activity is regulated in response to stress, and that mutations affecting GRed activity would have deleterious consequences. The recycling pathway for GSH formation is thus fundamental in the metabolism of GSH-dependent defense reactions [99]. In conclusion, the presence of GSH is essential, but not in itself sufficient, to prevent the cytotoxicity of ROS, being of fundamental importance the functionality of the glutathione-dependent enzymes, which participate in the first and second lines of defense (Fig. 1). Moreover, several experimental data indicate the tight interrelation between endogenous polyphenols, namely, GSH and its related enzymes, and dietary polyphenols [100].

5. Polyphenols and GSH-related enzymes

Several papers have described the effects of different classes of polyphenols on γ-GCS activity in cell cultures. Treatment of hepatoma cell line HepG2 with different plant-derived compounds increased γ-GCS activity and the consequent concentration of GSH [101]. A recent paper demonstrated that epicatechin and epicatechin gallate were able to counteract the strong decrease in GSH concentration and GRed activity in PC12 cells treated with Pb^{2+}. Surprisingly, the treatment of the cells with epigallocatechin gallate following Pb^{2+} exposure significantly reduced GSH concentration and GRed activity, which suggests that the effects of tea catechins on intracellular thiol status may be related to their chemical structures or the regulation of different gene expressions [102]. Other data, reporting on the effects of epigallocatechin gallate and ardisin on rat hepatocytes damage induced by oxidative toxic xenobiotics, or of catechin against UVB-induced skin damages, indicate that the flavonoids are able to activate the cellular antioxidative system by modulating the expression of some antioxidant enzymes [103,104]. The literature reports controversial results on the effect of polyphenol treatment on antioxidant enzyme activities that can differ among different cells or pathological conditions [105–107]. Several studies show, however, a positive effect of different classes of polyphenols on several enzyme activities, for example, GPx, SOD or GRed activities. This was the case with rat kidney exposed to red wine polyphenols or soy isoflavones, which induced a significant increase in several antioxidant enzyme activities [108–110]. Another flavonoid, silymarin, counteracted pancreatic damage in Wistar rats with alloxan-induced diabetes mellitus, by preventing the decrease in pancreatic antioxidant enzyme activities—GPx, SOD and catalase—caused by alloxan. In addition, silymarin produced by itself a significant increase in the activity of GPx after 15 days of administration to healthy rats [111]. Similar effects were obtained on hepatic GPx, GRed, SOD and catalase activities of mice chronically exposed to ethanol after pretreatment with quercetin for 15 days. The increase in enzyme activities was accompanied by a significant increase in GSH concentration and decrease in lipoperoxidation. The protective effects were not observed when mice were treated with quercetin after the chronic exposure to ethanol [112]. We have demonstrated that oleuropein and protocatechuic acid—phenolic compounds contained in extra virgin olive oil—remarkably increased GRed and mainly GPx activities in murine macrophage J774A.1 cells. It is worthy of note that in the same study we showed that the biophenols exerted a direct effect on DNA transcription of GRed and even more of GPx in J774A.1. We also described that J774.A1 treated with biophenols became unable to oxidize LDL probably through the strengthening of endogenous antioxidant enzymes and the increase of GSH with respect to the untreated cells. The observed protection exerted on murine macrophages and the parallel inhibition of cell-mediated LDL oxidation by the biophenols seemed to involve more than one mechanism [113]. These effects of polyphenols on the transcription of several enzyme genes associated to the antioxidant response were in agreement with previous in vitro studies on the anticarcinogenic and antithrombotic activities of several phenol compounds [114–117]. An interesting paper on human prostate transformed cell lines, LNCaP and PC-3, showed, by microarray, that soy isoflavons, in particular genistein, could modulate several genes, among which GPx gene was the most up-regulated [118]. The anticarcinogenic activity of several polyphenols could be due to the reinforcement of both endogenous antioxidant defenses and detoxifying activities [119] besides estrogenic/antiestrogenic activity, antiproliferation, cell cycle arrest and apoptosis, cellular signaling changes and regulation of the immune system [75]. Some studies point to the induction of GST as one of the principal anticarcinogenic mechanisms of different polyphenols sharing the 1,4-diphenol structure, also responsible for the strongest antioxidant activity [75,120]. As for the role of GST in cancer, however, there are controversial opinions. These enzymes show an increased activity in transformed cells and are considered to be involved in cellular multidrug resistance, an important form of clinical drug resistance to chemotherapeutic agents [121], as well as in the modulation of the apoptotic activity of malignant cells [122]. Recent papers have demonstrated that different wine and black tea polyphenols, as well as several flavonoids, for example, quercetin, kaempferol, luteolin, and others, modulate glutathione-related gene expression in colon tumor cells or in breast cancer cells, in particular reducing the expression of GST [123,124]. It

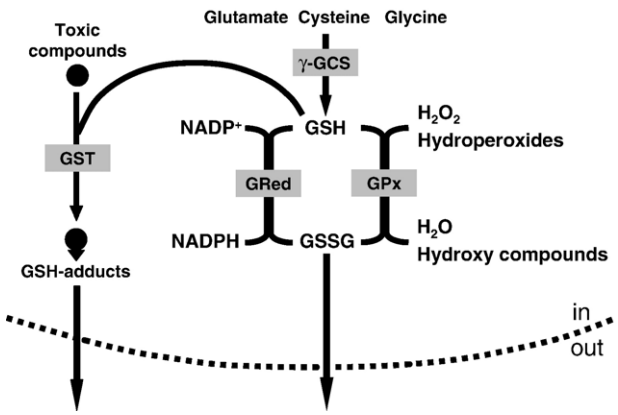

Fig. 1. Glutathione and related enzymes. Glutathione can directly scavenge free radicals or act as a substrate for GPx's and GSTs during the detoxification of hydrogen peroxide, lipid hydroperoxides and electrophilic compounds. During GST-mediated reactions, GSH is conjugated with various electrophiles, and the GSH adducts thus formed are actively secreted by the cell. The production of GSSG by GPx can lead to (i) the formation of mixed disulfides in cellular proteins, (ii) the release of GSSG excess by the cell, to maintain the intracellular GSH/GSSG ratio, or (iii) the back-reduction to GSH by GRed utilizing NADPH as a reductant. The resulting depletion of cellular GSH can be replaced by a de novo synthesis through two sequential ATP-dependent reactions catalyzed by γ-GCS—the rate-limiting enzyme—and glutathione synthetase.

should be pointed out that the effect of each polyphenol on a particular cell activity is difficult to predict [125]. In any case, recent papers have reported consistent structure–function relationships where the structure can affect bioavailability, antioxidant capacity and ability to induce antioxidant/detoxifying enzymes [124,126,127]. As reported up to now, several lines of evidence suggest a tight connection between exogenous and endogenous antioxidants, which appear to act in a coordinated fashion. It is reasonable to hypothesize that the coordination of the endogenous and exogenous antioxidant response is achieved, at least in part, through antioxidant responsive elements (AREs), which are found in the promoters of many of the genes that are inducible by oxidative and chemical stress, suggesting that they function in an interdependent integrated fashion.

6. Antioxidant responsive elements and the regulation of phase II enzymes

The transcriptional activation of phase II detoxifying and antioxidant enzymes that include NADPH (quinone oxidoreductase, γ-GCS, GST, GRed, GPx, sulfotransferases, epoxide hydrolases and other enzyme superfamilies, and/or antioxidant genes) has been related to *cis*-acting elements, detected in the promoter region of those genes. They regulate either or both constitutive and inducible gene expressions [128,129] and are called AREs or electrophile response elements (EpREs). Analyses of promoter regions suggest that several gene response elements may be involved in transcriptional regulation, including xenobiotic response elements and AREs/EpREs [130,131]. Antioxidant/electrophile response element sequences share a common motif, the so-called core sequence, which alone is not enough to mediate induction and needs a second core-like sequence adjacent to the primary one [132]. There is a fair amount of evidence that ARE/EpRE sequences play a pivotal role in the regulation of the cellular defense system, being in turn strictly regulated by transcriptional factors, such as the E2-related factors Nrf1 and mainly Nrf2, ubiquitously expressed and belonging to the basic region leucine zipper superfamily [133] (Fig. 2). Gel shift experiments showed that Nrf2 protein binds strongly to the ARE sequence and positively regulates its activity [134]. The interaction between Nrf2 and ARE also involves several inhibiting or activating cofactors. It was demonstrated that Kelch-like ECH-associated protein1 (Keap1)—bound to actin protein and localized in the perinuclear space—sequesters Nrf2 in the cytoplasm by forming heterodimers and, inhibiting its translocation to the nucleus, makes it unable to activate the ARE sequences [135,136]. The modulation of Keap1–Nrf2 binding seems to be a central event in the cellular response to oxidative stress, although the exact mechanism of dissociation of Nrf2 from its inhibitor, as well as the signal transduction pathway from oxidants to Nrf2–Keap1, remains largely unknown [137].

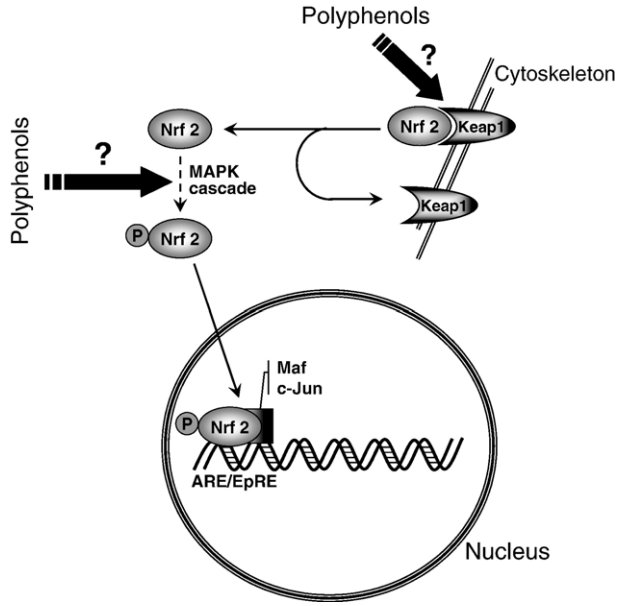

Fig. 2. Polyphenols induce phase II gene expressions through ARE activation. Polyphenols induce phase II genes by influencing the pathways that regulate ARE/EpRE activation. Polyphenols may (i) modify the capability of Keap1 in sequestering Nrf2 and/or (ii) activate MAPK proteins (ERK, JNK and p38), probably involved in Nrf2 stabilization. Nrf2 would thus translocate in the nucleus where it would transactivate the ARE/EpRE-containing promoter of phase II genes.

Although the involvement of superoxides and electrophiles as possible messengers in the oxidative stress pathway has not been demonstrated, there is some evidence [138] that they, presumably passing through unknown cytosolic factors, may help regulate Nrf2 released from Keap1 and its subsequent translocation into the nucleus. Nrf2 heterodimerizes with c-Jun or Small Maf or other unknown partners, inducing ARE activation and the consequent transcription of detoxifying enzyme genes [139–142]. Phosphorylation seems to be a principal mechanism in Nrf2 stabilization and involves several signal transcription pathways such as the MAPK, PKC and PI3K pathways [143–146]. As a consequence of kinases activation, Nrf2 dissociates from Keap1 and translocates in the nucleus where Nrf2 protein accumulates. However, there exist different signaling model systems on the upstream kinase signaling pathways. It is therefore possible that the transcriptional activity of Nrf2 be regulated by multiple converging signaling pathways, possibly regulated by upstream regulatory mechanisms, maybe chemical and cell type-dependent.

7. Polyphenols and ARE/EpRE elements

Interesting data were recently published by Myhrstad et al. to demonstrate that the increase in GSH levels found in COS-1 and HepG2 cells treated with quercetin or onion extract depends on an increased transcription of γ-GCS gene. More importantly, by the cells transfected with

luciferase reporter constructs consisting of fragments of the GCS promoter containing different ARE/EpRE sequences, it was demonstrated that the observed increase in gene transcription is mediated by the activation of antioxidant response elements in the gene promoter [147,148]. These results are in agreement with previous data regarding quercetin effects on human NADPH:quinone oxidoreductase gene expression in a human breast carcinoma cell line [149]. Flavonoids, proanthocyanidins and specific flavonol and catechins contained in extracts from Mauritian endemic plants showed modulatory effects on promoter activities of several antioxidants enzymes in COS7 monkey renal tubular cells. Interestingly, while the promoter activity of SOD was associated with increased phenolic content, that of GPx was inversely related to proanthocyanidins content [150]. It has been also demonstrated that compounds having light structural differences exhibited a markedly different efficiency in inducing phase II enzymes [124,151]. In the same vein are the results obtained with five different green tea catechins on HepG2-C8 cells [152]. The catechins showing the strongest activity in inducing phase II gene expressions through ARE activation were (−)epigallocatechin-3-gallate and (−)epicatechin-3-gallate, indicating the efficacy of the 3-gallate group. Several experimental data strongly indicate that dietary polyphenols can stimulate the transcription of antioxidant and detoxification defense systems through ARE elements. Moreover, the different efficiency showed by polyphenol structures clearly indicate a strong structure–activity relationship that may be related to the antioxidant capacity of each compound or to the different capacity to act as ligands for receptors unknown so far. It must be also taken into account that the degree of oxidative stress, the polyphenol concentration as well as the biological system studied introduce other elements of variability in the observed response. A further hypothesis could be that polyphenols influence the pathways that regulate ARE/EpRE activation, by modifying the capability of Keap1 in sequestering Nrf2. Nrf2 would thus be released and would translocate in the nucleus and transactivate the ARE/EpRE-containing promoter of several genes. This hypothesis is supported by the evidence that polyphenols can react with active sulfhydryl groups [153], closely related to the enzyme induction and GSH increase [154], thus modulating several sensor proteins, Keap1 among others [155]. It should be also taken into account that phenolic antioxidants can influence ARE-dependent gene expression through the activation of MAPK proteins (ERK, JNK and p38), probably involved in Nrf2 stabilization through its phosphorylation [156]. Green tea polyphenol extracts stimulate the transcription of phase II enzymes by ARE activation probably utilizing the MAPK signaling pathway [157]. More recently, (−)epigallocatechin-3-gallate showed potent activation of all three MAPKs in a dose- and time-dependent manner, whereas (−)epicatechin-3-gallate activated ERK and p38 [158]. The same was demonstrated by topical treatment of UV-irradiated SKH-1 hairless mice with green tea polyphenols [116]. Resveratrol, considered as an active cardioprotective component of red wine, enhanced the activity of phase II enzymes [159,160]. It would be reasonable to hypothesize that resveratrol up-regulates phase II antioxidant/detoxifying enzymes through Nrf2 activation, probably mediated by MAPKs or PKB/Akt. It has been in fact demonstrated that resveratrol is able to activate MAPKs in human melanoma [161] and mouse epidermal JB6 cells [162], and PKB/Akt in MCF-7 cells [163]. It must be considered, however, that the phenol compounds can selectively activate or inhibit different protein kinases depending on their concentration and cell types.

8. Conclusions

Although most polyphenols have antioxidant properties, these properties alone may not account for all of their beneficial effects. Emerging findings suggest a variety of potential mechanisms of action of polyphenols in cytoprotection against oxidative stress, which may be independent of conventional antioxidant-reducing activities. Such mechanisms might entail the interaction of polyphenols with cell signaling and influence gene expression, with the consequent modulation of specific enzymatic activities that drive the intracellular response against oxidative stress. In any case it is important to highlight that the majority of the reported studies were performed in vitro. It is thus mandatory to confirm those findings by in vivo studies so as to obtain useful information for eventual therapeutic or dietary interventions.

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