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Dose-dependent functionality and toxicity of green tea polyphenols in experimental rodents

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ABSTRACT

A large number of physiologically functional foods are comprised of plant polyphenols. Their antioxidative activities have been intensively studied for a long period and proposed to be one of the major mechanisms of action accounting for their health promotional and disease preventive effects. Green tea polyphenols (GTPs) are considered to possess marked anti-oxidative properties and versatile beneficial functions, including anti-inflammation and cancer prevention. On the other hand, some investigators, including us, have uncovered their toxicity at high doses presumably due to pro-oxidative properties. For instance, both experimental animal studies and epidemiological surveys have demonstrated that GTPs may cause hepatotoxicity. We also recently showed that diets containing high doses (0.5–1%) of a GTP deteriorated dextran sodium sulfate (DSS)-induced intestinal inflammation and carcinogenesis. In addition, colitis mode mice fed a 1% GTP exhibited symptoms of nephrotoxicity, as indicated by marked elevation of serum creatinine level. This diet also increased thiobarbituric acid-reactive substances, a reliable marker of oxidative damage, in both kidneys and livers even in normal mice, while the expression levels of antioxidant enzymes and heat shock proteins (HSPs) were diminished in colitis and normal mice. Intriguingly, GTPs at 0.01% and 0.1% showed hepato-protective activities, *i.e.*, they significantly suppressed DSS-increased serum aspartate aminotransferase and alanine aminotransferase levels. Moreover, those diets remarkably restored DSS-down-regulated expressions of heme oxygenase-1 and HSP70 in livers and kidneys. Taken together, while low and medium doses of GTPs are beneficial in colitis model mice, unwanted side-effects occasionally emerge with high doses. This dose-dependent functionality and toxicity of GTPs are in accordance with the concept of hormesis, in which mild, but not severe, stress activates defense systems for adaptation and survival.

Stress-induced biomolecule modifications

Homeostatic regulation of biological functions of macromolecules, such as membrane lipids, enzymes, proteins, and DNA, is a key determinant for health promotion, disease prevention, and longevity. Reactive oxygen species (ROS)[1] have long been demonstrated to play numerous roles in both physiological and pathological conditions *via* oxidative modification of biomolecules present in cells and tissues. Accumulation of oxidative damage footprints in those biological molecules has been described to be involved in the onset of numerous diseases and aging [1]. In particular, biochemical modifications of cellular DNA [2] and proteins [3] by ROS and lipid peroxidation products, such as acrolein [4], malondialdehyde [5], 4-hydroxy-2-nonenal [6] lead to either loss or undesired gain of their functions for homeostasis disruption (Fig. 1). In addition, mitochondrial DNA mutations caused by ROS play significant roles in dysfunctions of cellular metabolism, and defense and other systems [7], though Laqouge and Larsson have proposed that those mutations may be generated by replication errors rather than accumulated oxidative damage [8]. Recently, Akatsuka et al. uncovered a novel base modification, *i.e.*, 1,N^6-propanoadenine (acrolein–adenine), in a ferric nitrilotriacetate-treated mouse model, in which oxidative stress plays an essential role in the development of renal carcinogenesis [9]. Also, mutations of the *Cu,Zn-sod* gene were reported to partially truncate anti-oxidative systems, thereby increasing the risk of familial

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[1] *Abbreviations used*: ABC, ATP-binding cassette; ACR, acrolein; ALS, amyotrophic lateral sclerosis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DMH, dimethylhydrazine; DSS, dextran sulfate sodium; EGCG, (-)-epigallocatehin-3-gallate; ERK, extracellular signal-regulated kinase; GTP, green tea polyphenols; GSH, glutathione; GST, GSH *S*-transferase; HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase-1; Hsf, heat shock factor; HS, heat shock; HSP, heat shock protein; Keap1, Kelch-like ECH-associated protein; MDA, malondialdehyde; Nrf2, nuclear factor (erythroid-derived 2)-like 2; NQO1, NAD(P)H dehydrogenase; PQC, protein quality control; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; Ub, ubiquitin.

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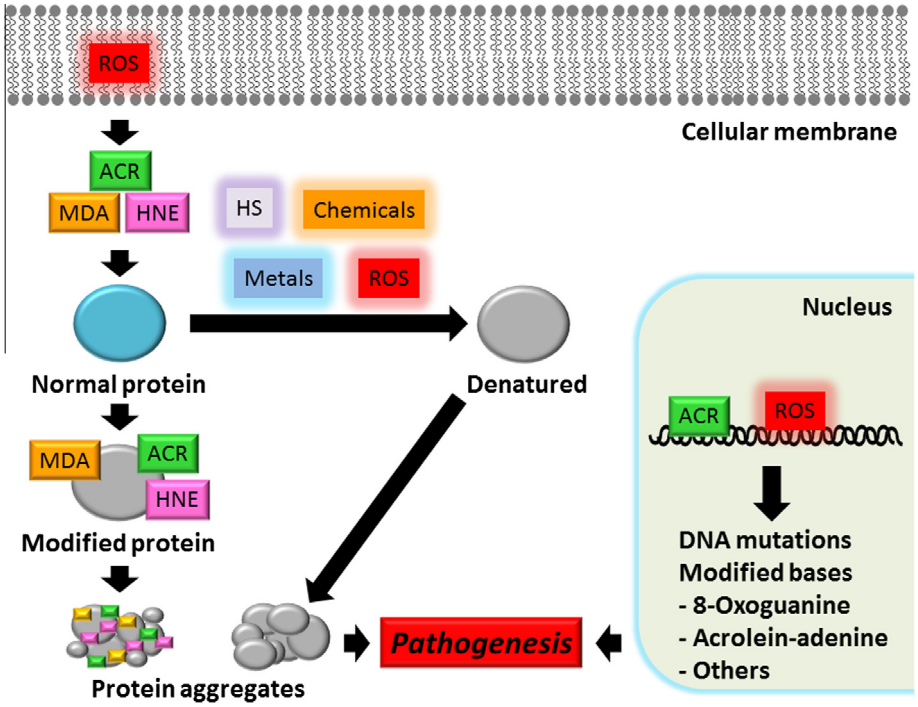

Fig. 1. Modifications of cellular membrane lipids, proteins, and DNA by reactive oxygen species and electrophilic aldehyde compounds, the latter of which are degradation products of lipid peroxide. ROS, reactive oxygen species; ACR, acrolein; MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; HS, heat shock.

amyotrophic lateral sclerosis (ALS) [10], a neurodegenerative disease that exhibits dysfunctions of muscle movement and strength. On the other hand, proteo-stresses have been shown to be characterized to be inducible by physical, chemical, and biological stimuli, and interfere with and disable protein folding for denaturation [11]. Other recent studies have demonstrated that accumulation of un- and mis-folded proteins may promote the formation of their aggregates, the hallmark of neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's diseases [12]. In addition, the status of cellular junk proteins is associated with adiposity and thus the development of metabolic syndrome [13]. Furthermore, it should be pointed out that concerted and tight regulation of the biological protein quality may contribute to longevity, as demonstrated in experiments using the naked mole rats, which have marked protein stability and a long life-span [14]. Thus, it is highly conceivable that quality maintenance of biological proteins greatly affects health status and longevity. Among a number of constitutive and inducible mechanisms that function to protect biological macromolecules from oxidative stress and proteo-stress, 2 representative biochemical defense systems are highlighted (Fig. 2) and described below.

The Keap1/Nrf2-dependent defense system

Anti-oxidation can be defined as a self-defense mechanism ubiquitously distributed among organisms. Anti-oxidative modes of actions can be classified into at least the following 3 categories: (1) scavenging and quenching ROS [as seen with vitamin C, vitamin E, glutathione (GSH), *etc.*], (2) attenuation of ROS-generating enzymes (NADPH oxidase and xanthine oxidase, *etc.*), and (3) up-regulation of anti-oxidant enzymes [superoxide dismutase (SOD), catalase, *etc.*]. The Kelch-like ECH-associated protein 1/nuclear factor (erythroid-derived 2)-like 2 (Keap1/Nrf2) system, belonging to the category (3), adaptively functions to protect cells from endogenous and exogenous oxidative and electrophilic damages [15] (Fig. 3). In a normal state, the transcription factor Nrf2 is continuously ubiquitinated by the Cul3-Keap1 ubiquitin E3 ligase complex and thereby rapidly transported to degradation systems in proteasomes. ROS and electrophilic chemicals oxidize the reactive cysteine residues of Keap1 in both direct and indirect manners. This critical step stabilizes Nrf2, thereby inducing robust expressions of a battery of cytoprotective genes, including those related to anti-oxidation [Cu/Zn-SOD, Mn-SOD, extracellular SOD, γ-glutamylcysteine synthetase, *etc.*], xenobiotic detoxification [GSH *S*-transferase (GST), glucuronidase, sulfatase, *etc.*], and protein quality control (PQC) (molecular chaperones, ubiquitin/proteasome systems, *etc.*) for adaptation [15]. In addition, prior to translocation of Nrf2 into the nucleus, its transcription activity is modulated by several protein kinases, which are simultaneously activated by stressors. Feng et al. disclosed that activation of Akt and extracellular signal-regulated kinase (ERK)1/2 is required for activation of Nrf2, leading to up-regulation of the expression of heme oxygenase (HO)-1, one of the major inducible anti-oxidant enzymes [16]. On the other hand, Nrf2 activity is continuously repressed in a normal state by Bach1, which constitutively binds to the Maf recognition element [17].

Heat shock proteins as molecular chaperones

Various types of stresses toward biological proteins critically disrupt their conformation and folding state, which often results in abolishment of their biological functions. A number of recent studies have indicated that several distinct PQC systems play key roles in counteraction against proteo-stress. Heat shock proteins (HSPs), highly conserved families of proteins ubiquitously expressed in most types of cells, are molecular chaperones that allow misfolded and unfolded proteins to achieve functionally active conformation (Fig. 4). The expression and functional status of HSPs are considered to be critical determinants of PQC systems and thus essentially associated with homeostasis, health and longevity. In fact, expression of HSPs at high levels substantially contributes to extending the lifespan of many experimental animals [18]. HSPs are comprised of numerous family proteins.