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Modulation of Protein Quality Control Systems as Novel Mechanisms Underlying Functionality of Food Phytochemicals

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ABSTRACT

Background: Phytochemicals are secondary metabolites of plants that are produced for their defense against environmental stresses, such as polyphenols, which are considered to play a major role in protection against ultraviolet (UV) light-induced oxidative damage, as well as anti-fungal and anti-microbial activities. In addition, there is a great body of evidence showing that phytochemicals exhibit a wide array of physiological activities in humans. Accumulated data show that the bioavailability of most, if not all, phytochemicals is quite poor and their substantial biotransformation after ingestion has also been noted. Thus, they are characterized as non-nutritive xenobiotics in animals, and the question of why phytochemicals, which are produced for plant self-defense, have beneficial effects in humans is quite intriguing. Meanwhile, stress-induced denaturing of cellular proteins greatly affects their tertiary structure and critically disrupts their biological functions, occasionally leading to aggregation for the onset of some pathology. Many recent studies have indicated that protein quality control (PQC) systems play key roles in counteracting ‘proteo-stress’, which is comprised of several processes, including protein refolding by heat shock proteins (HSPs) and degradation of abnormal proteins by the ubiquitin-proteasome system as well as autophagy.

Objective: Phytochemicals are xenobiotics, thus their biochemical interactions with animal proteins are considered to occur in a non-specific manner, which raises the possibility that some phytochemicals cause proteo-stress for activating PQC systems. Because their status is thought to be a critical determinant of homeostasis, the physiological functions of phytochemicals may be partially mediated through those unique systems. The present study was thus undertaken to address this possibility.

Methods and Results: We focused on zerumbone (ZER), an electrophilic sesquiterpene present in *Zingiber zerumbet* Smith (shampoo ginger). This agent has been reported to exhibit various bioactivities, including anti-inflammation and cancer prevention[1,2]. Treatment of Hepa1c1c7 mouse hepatoma cells with ZER resulted in marked up-regulation of multiple HSPs, such as HSP40 and HSP70. Furthermore, oral administration to the nematode *Caenorhabditis elegans* and SD rats increased the expressions of some HSPs[3]. Interestingly, ZER also increased proteasome activity in Hepa1c1c7 cells, which was accompanied with up-regulation of β5, a major proteasome functional protein. In addition, the agent notably up-regulated the expressions of several pro-autophagic markers, including p62 and microtubule-associated protein 1 light-chain 3 (LC3)-II[4]. Experiments with biotin-labeled ZER as well as a specific antibody against ZER-adduct proteins revealed that it binds numerous cellular proteins in a non-specific manner. Along a similar line, incubation with ZER led to formations of p62-conjugated proteins and aggresomes. Together, these results suggest that ZER causes proteo-stress for potentiating the integrity of PQC systems. In support of this notion, ZER-bound proteins have been suggested to be partially recognized by HSP90, leading to dissociation of heat shok factor 1 (HSF1) from HSP90 for inducing multiple HSP genes. Next we speculated that mild chemical stress by ZER may exert beneficial effects, since ZER-bound proteins were time-dependently degraded, suggesting that defense capacity was amplified to a great level as compared with the non-treated condition. As expected, ZER conferred thermoresistance to *Caenorhabditis elegans* (*C. elegans*) and suppressed the proteo-toxicity of 4-hydroxy-2-nonenal, a potent electrophile produced through a lipid peroxidation process, in a p62-dependent manner. We then screened a number of nutrients and phytochemicals for their HSP70 inducibility, and found that certain phytochemicals, such as curcumin, phenethyl isothiocyanate, ursolic acid, and lycopene, were significantly active, whereas most nutrients were virtually inactive. These results may be associated with the fact that phytochemicals, but not nutrients, are foreign chemicals to animals, as noted above.

Conclusion: Up-regulation of antioxidant and xenobiotics-metabolizing enzymes has been reported to be an adaptive response in animals exposed to phytochemicals. Our present results imply that the process also increases the capacity to counteract proteo-stresses through activation of PQC systems. This putative phenomenon, representing the concept of hormesis[5], may be associated with mechanisms underlying the physiological functions of phytochemicals. Therefore, chronic ingestion of this class of chemicals may result in 'chemical training', in which self-defense systems are continuously activated for adaptation to phytochemical-driven stresses.

Key words: heat shock proteins, ubiquitin-proteasome system, autophagy, *C. elegans*