Forum

Role of Dietary Supplements/Nutraceuticals in Chemoprevention through Induction of Cytoprotective Enzymes

Jed W. Fahey[†,‡,§] and Thomas W. Kensler*[,†,||]

Departments of Pharmacology and Molecular Sciences, International Health, and Environmental Health Sciences, Johns Hopkins University, Baltimore, Maryland 21205

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Rationale for Enzyme Induction as a Chemopreventive Strategy

Numerous epidemiological studies from many parts of the world report strikingly lower cancer risks among individuals who consume large quantities of fruits and vegetables (reviewed in refs *1* and *2*). As a consequence, a great variety of foods and supplements have been implicated as being sources of protective phytochemical factors. These factors can be used as discrete chemicals, dietary supplements, or functional foods. Others have contrasted the fact that dietary supplements are generally considered to be time-tested but in large part scientifically unproven, whereas functional foods are components of the normal diet that are increasingly shown to have inherent value for maintaining human health (*3*). The popular literature repeatedly highlights some of these phytochemicals more than others [e.g., capsaicin from peppers, coumarins from citrus and tomatoes, epigallocatechin-3-gallate (EGCG) from green tea, genistein from soybeans, indoles from broccoli and cabbage, isothiocyanates from cruciferous vegetables, lycopene from tomatoes and red grapefruit, allicin from garlic and onions, triterpenoids from licorice root and citrus, pectin from grapefruit, resveratrol from grapes, carotenoids from red grapefruit, quercetin from onions and broccoli, and flavonoids from a variety of fruits and vegetables]. The mechanisms responsible for the possible protective effects derived from the consumption of these foods are multiple, probably involve complex interactions, and are incompletely understood. Nonetheless, there is extensive literature detailing the actions of specific phytochemicals as well as the intact plants themselves toward altering the expression of genes that reflect an adaptive stress response, which enhances normal cell survival, and may also promote apoptosis or cell cycle arrest in tumor cells. In the face of electrophilic and oxidative insults, these inducible gene products have been shown to facilitate the conjugation of xenobiotics and the nucleophilic trapping of activated electrophilic xenobiotics and to increase the overall antioxidative capacity in cells, animals, and in some cases humans (*4*−*8*). Moreover, substantive experimental evidence in animals has been developed to support the view that the coordinated induction of these cytoprotective enzymes is a critical and sufficient mechanism to engender protection against the toxic and carcinogenic actions of reactive intermediates (reviewed in refs *6* and *9*). As a consequence, monitoring for inducers of this stress response can be an informative means to identify plants and constituent phytochemicals of potential chemopreventive utility.

Table 1. General Structural Classes of Natural Compounds Inducing Chemoprotective Enzymes

general class	recent review
diphenols, phenylenediamines, and quinones	*10*
Michael reaction acceptors	*10*
glucosinolate-derived isothiocyanates (e.g., sulforaphane), dithiocarbamates (e.g., brassinin), and indoles (e.g., indole-3-carbinol [I3C] and di-indolymethane [DIM])	*10*
aliphatic sulfides (including 1,2-dithiole-3-thiones and oxathiolene oxides)	*10*
carotenoids and other conjugated polyenes such as chlorophyll	*10*
terpenoids (including triterpenoids)	*10, 42*
ceramides	*42*
withanolides	*42*
flavonoids (including flavones, flavanones, flavonols, isoflavones, and chalcones)	*10, 42*
alkaloids	*42*
diaryl heptanoids	*42*
phenylpropenoids	*43*

Chemical Classes of Inducers. A great deal of effort has gone into the identification of structural moieties that induce chemoprotective enzymes, and this structure−inducer potency data have recently been reviewed (*10*). Some of these protective enzyme-inducing phytochemicals, for example, the glucosinolate derivative isothiocyanates and indoles, are found exclusively in broccoli and its close relatives. Others, such as the carotenoids, flavonoids, and chlorophyll, are of almost universal provenance in green plants, with antioxidant, anti-inflammatory, antiallergic, anticarcinogenic, and even antiviral activities having been ascribed to them (*11*, *12*). The major structural classes of naturally occurring inducers are summarized in Table 1. Most, but not all of them, are phytochemicals. In our work, we have given special attention to the glucosinolates and isothiocyanates from *Brassica* vegetables such as cauliflower, Brussels sprouts, broccoli, and cabbage (*13*); to chlorophyllin (*14*−*16*), which

* To whom correspondence should be addressed. Tel: 410-955-4712. Fax: 410-955-0116. E-mail: tkensler@jhsph.edu.
[†] Department of Pharmacology and Molecular Sciences.
[‡] Department of International Health.
[§] Dr. Fahey, as well as Johns Hopkins University, owns stock in Brassica Protection Products, LLC (BPP), a company whose mission is to develop chemoprotective food products and which sells broccoli sprouts. Dr. Fahey is an unpaid scientific consultant to BPP, and his founder's stock is subject to certain restrictions under University policy. The terms of this arrangement are being managed by Johns Hopkins University in accordance with its conflict of interest policies.
[||] Department of Environmental Health Sciences.

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can be derived from extracts of virtually any green plant; and to certain flavonoids (*17*) and triterpenoids (*5*). To identify and isolate inducers of chemoprotective enzymes, we and others have utilized the Prochaska microtiter plate NQO1 assay [which measures the induction of quinone reductase, one of the quintessential detoxification enzymes in the adaptive stress response (*18*, *19*)]. Guided by this approach, sulforaphane was isolated from broccoli in 1992 (*20*). The use of this bioassay has also guided studies of structure−activity relationships, the synthesis of analogues, elucidation of mechanisms, and assessment of potencies (*21*).

Mode of Action. Multiple defense systems have evolved in all multicellular organisms in order to ensure protection against the toxic effects of the plethora of endogenous and exogenous oxidants and electrophiles to which they are exposed. DNA damage resulting from these reactive intermediates is an integral component of carcinogenesis, and strategies to reduce the burden of damage to the genome have been clearly shown to prevent cancer development in animals (*6*, *22*). There exist several signaling pathways that evoke an adaptive response to the stress elicited by oxidants and electrophiles. A key pathway is that of Keap1−Nrf2−ARE signaling. In mice, disruption of this pathway dramatically alters the response of animals to chemical carcinogens, oxidants, inflammatory states, and other toxins targeting a variety of organs (*7*). Induction of this cytoprotective response system requires at least three essential components: (i) antioxidant response elements (AREs) (*23*), upstream regulatory sequences present on each responsive gene in either single or multiple copies; (ii) Nrf2, the principal transcription factor that heterodimerizes with members of the small Maf family of transcription factors, binds to the ARE, and recruits the general transcriptional machinery for expression of ARE-regulated genes (*24*); and (iii) Keap1, a cytosolic repressor protein that binds to Nrf2, retains it in the cytoplasm, and promotes its proteasomal degradation (*25*). Inducers react with thiols in Keap1 at rates that are closely related to their potencies, leading to disruption of the Nrf2−Keap1 complex and nuclear accumulation of Nrf2 (*26*). The molecular features of this pathway have been recently reviewed in *Chemical Research in Toxicology* (*27*) and elsewhere (*28*), but it is already clear that phytochemicals such as sulforaphane directly interact with Keap1 to trigger a gene expression response (*29*).

Sources and Delivery of Cytoprotective Agents. Extracts of plants and isolated natural products from those plants exhibit a bewildering array of activities in bioassays and in animal models, and we are beginning to see such products subjected to rigorous scrutiny in human volunteers. The induction of the cytoprotective enzymes has now been widely studied in cell culture and in animal models, and clinical investigations are beginning to unfold (*30*). A wealth of information exists on cell-based assays that have good predictive value, at least to the stage of animal models of carcinogenesis. An interesting conclusion that can be drawn from this body of literature is as follows: There are a number of potent, naturally occurring inducers of protective enzymes that also have low cytotoxicity, (e.g., sulforaphane, resveratrol, pinostrobin, and EGCG). By our calculations, consumption of a serving or two of foods like broccoli sprouts (sulforaphane glucosinolate) or Thai ginger (pinostrobin) can be expected to be effective in inducing the chemoprotective enzyme response (*17*, *31*). For example, we recently conducted a clinical study in China using standardized doses of broccoli sprout-derived sulforaphane glucosinolate and observed a dose-dependent reduction in markers of DNA damage (*32*). Levels of sulforaphane were calibrated to be

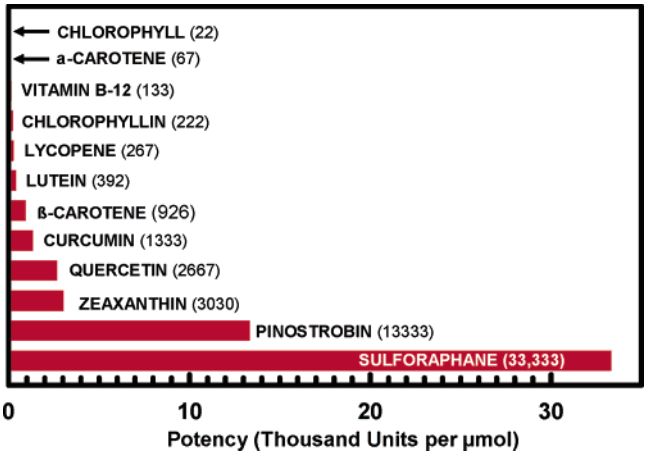

Figure 1. Induction of the chemoprotective enzyme NQO1 by phytochemicals in cell culture. Bioassay data were adapted from refs *16*, *17*, and *44*.

achievable by dietary means. However, the consumption of quite high levels of the plants in which some of these compounds are found would be necessary to achieve what can be reasonably expected to be a protective, but not toxic, level of active phytochemical. When calculations are made of the quantity of certain other potentially protective phytochemicals (e.g., from wasabi or mustard), achieving an efficacious dose does not appear to be possible (or advisable) by dietary means.

This presents a conundrum. Should persons at increased risk for particular cancers be encouraged to take supplements or food products enriched in potentially protective compounds? There is tremendous variation in the inducer potency of these compounds (Figure 1). The consumption of a varied diet rich in potentially protective foods (Table 2) has clear attraction from the perspective of safety (low risk of overdosing or toxicity). On the other hand, the evolution of modern humans' diet has been phenomenally rapid over the past few generations (Figure 2) and an argument can be made that dietary supplementation may be called for if we are to achieve a more uniformly enhanced longevity across populations—an incompletely understood phenomenon, which is now enjoyed by a fraction of individuals in Western and other populations. Thus, we have also approached the issue of substances that have a very high safety index (e.g., one can consume a lot of it without adverse effects) but relatively low inducer potency, in an alternative fashion. We have examined compounds such as chlorophyllin and chlorophyll (*14*−*16*) and foods such as honey (*17*), which have very long and clear records of safe consumption but do not have particularly high potency as inducers of chemoprotective enzymes. Dogmatic approaches to protection frequently assume that one would need to attain high levels of protection in order for there to be value in this strategy. Whereas this may be the only paradigm that permits us to measure risk reduction using currently available tools, it also embodies the flawed logic that the only good risk reduction is a large risk reduction. Consider the following examples.

In the context of the amount of sugar consumed in economically advantaged countries (194 g/day of sugar is eaten by Americans) (*33*), we have determined that replacing a small fraction of this quantity with honey might well yield small but significant cancer protective results in addition to all of the other obvious benefits of replacing white sugar or high fructose corn syrup with honey (*17*). A similar argument can be made for chlorophyll and some of the accessory plant pigments (e.g., carotenoids and xanthophylls) that are present in essentially all

Table 2. Common Edible Plants and Plant Products that Induce Chemoprotective Enzymes

common name	scientific name	predominant enzyme-inducing phytochemical(s)
	Crucifer family	
arugula	*Eruca sativa*	isothiocyanates (e.g., erucin)
broccoli	*Brassica oleracea*	isothiocyanates (e.g., sulforaphane)
Brussels sprouts	*B. oleracea*	indole glucosinolate metabolites (I3C, DIM)
cabbage	*B. oleracea*	isothiocyanates (e.g., allyl-)
horseradish	*Armoracia rusticana*	isothiocyanates (e.g., allyl-)
mustard	*Sinapis* spp.	isothiocyanates (e.g., allyl-)
radish	*Raphanus sativus*	isothiocyanates (e.g., sulforaphene)
watercress	*Nasturtium nasturtium-aquaticum*	isothiocyanates (e.g., phenylethyl-)
wasabi	*Wasabia japonica*	isothiocyanates (e.g., allyl-)
	onion (Lily) family	
onion	*Allium cepa*	quercetin glycoside(s) + allyl sulfide
garlic	*Allium sativum*	allyl sulfide
	other	
banana	*Musa* spp.	bicyclic diarylheptanoid
green tea	*Camillia sinensis*	polyphenols (e.g., EGCG)
ginger	*Zingiber officinale*	flavonoids
tephrosia	*Tephrosia* spp.	flavonoids
Thai ginger (fingerroot)	*Boesenbergia pandurata*	flavonoids (e.g., pinostrobin)
tomatillos	*Physalis philadelphica*	withanolides
turmeric	*Curcuma longa*	curcumin

higher plants (fruits and vegetables) yet are only moderately to slightly potent inducers in the assay systems that have been employed (*16*). Thus, the additive effect of a multitude of weak to moderately potent inducers for people eating five or more servings a day of fruits and vegetables may be highly significant. Reducing the risk of cancer by only 1%, in this country alone, would equate to saving about 5600 lives. If one assumes that these lives saved resulted in an average of 15 years of additional life, this would account for 82500 life years saved (a frequently used metric) per year of dietary change or intervention. Thus, although it may be next to impossible to measure as small a risk reduction as 1 or 2% (placebo-controlled clinical trials with a tumor end point would be impossible to conduct), the significance of even this magnitude of preventive effect should not be lost on policy makers. The risks in this example are essentially nonexistent.

Conversely, there are risks in a supplementation or food fortification strategy [e.g., interactions with medications; reviewed recently by Ohnishi (*34*)]. These risks must be weighed against a higher real or possibly perceived ability to reduce the risk of a variety of chronic and degenerative diseases. This pot of gold at the end of the proverbial rainbow has lured a multitude of companies of all sizes and qualities to move into this business area. Many of the smaller companies make products rapidly. Some of them make claims that are allowed under the Food and Drug Administration (FDA) guidelines and create high quality products, some of them pose spurious claims or they ride the popular media's latest "cure-de-jour" publicity, and some make no attempt whatsoever to differentiate or distinguish their products. Many of the larger companies insist on rigorous review of claims language and insist on some degree of clinical trial support. As a result of this risk-averse positioning, they are frequently slow to create products in reaction to high profile scientific studies. When they do, however, there is almost always significant patent protection surrounding their product introductions, protecting either processes, formulations, or in some cases putative mode of action. Once issued, these patents create a formidable barrier to other companies because contesting or litigating against them is very costly and very difficult. Few small companies can afford such protracted litigation.

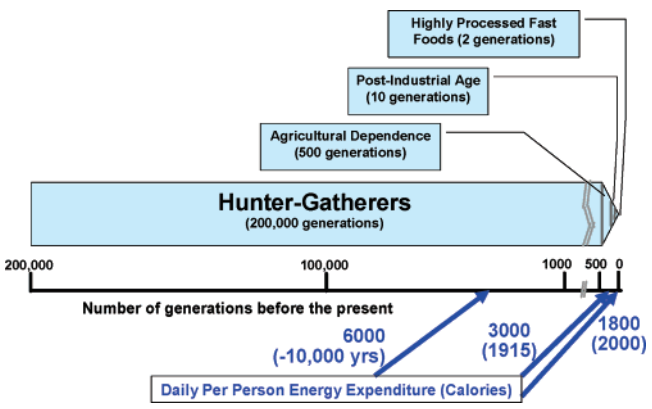

Figure 2. Human dietary experience.

Food vs Supplement. Most scientists have now come to accept the views of Doll and Peto (*35*) that a sizable fraction of cancer mortality is attributable to diet. However, acceptance of this epidemiologic reality leads to one of the most perplexing issues in nutrition education: Should we address the prevention of chronic disease primarily through encouraging better diet and more exercise or yield to the realities of reduced energy expenditure in the modern world and support the rational development of supplements that might not require, for example, the consumption of at least five servings per day of fruits and vegetables (*36*)? As already discussed (see Figure 2), the net energy expenditure in industrialized society has plummeted. It is now about half of what it was a century ago. This has led to what many people point out is a disconnect between dietary recommendations (e.g., eat five or more servings per day of fruits and vegetables) and a dramatically reduced caloric requirement for what has become a much more sedentary population than even its grandparents' generation. As a result, some make the case that it may be very difficult to maintain a healthy body weight *and* maintain an optimal "protected" state vis-à-vis intake of phytochemical inducers of chemoprotective enzymes plus the appropriate levels of vitamins and minerals. Are such people who wish to practice preventive behavior and control their weight candidates for pills or other concentrated doses of phytochemical supplements or for foods and food products reinforced with the same bioactive ingredients? Perhaps, should they be? We are unfortunately still at a state scientifically whereby it is exceptionally difficult to study experimentally, or even to model mathematically, the health effects of multiple phytochemicals. One must study the effect of an extract on animal or human metabolism, or one must

examine the effects of isolated active compounds. Experimentally, a combinatorial approach, even in cell culture, is problematic. There have been hints and anecdotes from many quarters of the effects of, for example, plant extracts or plant preparations, without concomitant efficacy of isolated components from those extracts. Globally, there is an abundance of both written and verbal anecdotal evidence on the efficacy of a huge variety of folk medicines—so much so that clearly there are effects worth pursuing with scientific rigor. To our knowledge, although much descriptive work has been generated, nobody has yet solved the issue of chemically dissecting the multitude of bioactive compounds from such medicines and biologically evaluating them in such a manner that proves the synergies rumored to exist in such concoctions, decoctions, preparations, elixirs, extracts, and tonics. Are there truly synergies or matrix effects? We have no answers to these questions but propose that they deserve to be the substrate for creative minds over the next decade.

Importance of Using Scientific Principles: Evidence-Based Medicine

There is tremendous appeal to the consumer for dietary supplements: perceived efficacy coupled with low cost, ease of distribution, culturally acceptability, and presumed safety. Globally, the business of dietary supplements is booming. In the United States, business has become unfettered by the deregulatory effect of the 1994 Dietary Supplement Health and Education Act (DSHEA). DSHEA precludes the FDA from regulating dietary supplements as a drug solely because of any statements on the product labels regarding health claims. Whereas the manufacturing and clinical evaluation of drugs is well-defined by the FDA approval process, there are no clear guidelines for creating or evaluating supplements. Despite the popularity of botanical dietary supplements, many of these materials are not well-characterized in terms of their mechanisms of action, toxicity, or efficacy in humans. Moreover, as discussed elsewhere in this forum, there has been a lack of standardization of the composition of some dietary supplements.

It is certainly our view, and one shared by much of the scientific community, that the development of chemopreventive foods (supplements and nutraceuticals) should follow a pathway designed to create knowledge about composition and pharmacology. There are a number of key issues that need to be considered. Unlike many botanical products where efficacy is inferred on the basis of limited or no information on mechanisms of action, the use of a molecular target such as Nrf2 signaling for identification and evaluation of chemopreventive foods provides a fast track to meeting the essential scientific requirements typical of drug development. Chang (*37*) and Talalay (*38*) have recently reviewed the critical issues underlying the development of either a plant extract or a whole plant to yield a product with a consistent pharmacological activity. Briefly, they include (i) quality control issues related to chemical composition (e.g., plant selection, source, constituents, and contaminants); (ii) standard protocols for formulation and testing (both chemical and pharmacological profiles); (iii) safety testing, which is usually assumed, but not proven; (iv) identification of mechanism-based biomarkers to evaluate beneficial/harmful effects; and (v) prospective clinical trials. Other pertinent reviews of this topic are also noted (*39*, *40*). While it is beyond the scope of this article to document application of these tenets to development of specific phytochemicals, supplements, or foods for cancer chemoprevention, the reader is referred to the literature regarding curcumin and sulforaphane (in glucosinolate-rich broccoli) as lead examples of the application of evidence-based approaches (*8*, *38*, *41*). Fulfillment of these tenets is essential in establishing the true value of food-derived chemopreventive interventions.

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