

## Review Article

# On Enzyme-Based Anticancer Molecular Dietary Manipulations

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Evidence from both epidemiological and experimental observations has fuelled the belief that the high consumption of fruits and vegetables rich in nutrients and phytochemicals may help prevent cancer and heart disease in humans. This concept has been drastically simplified from the dietary approaches to the use of single bioactive components both as a single supplement or in functional foods to manipulate xenobiotic metabolism. These procedures, which aim to induce mutagen/carcinogen detoxification or inhibit their bioactivation, fail to take into account the multiple and paradoxical biological outcomes of enzyme modulators that make their effects unpredictable. Here, we show that the idea that the physiological roles of specific catalysts may be easily manipulated by regular long-term administration of isolated nutrients and other chemicals derived from food plants is not viable. In contrast, we claim that the consumption of healthy diets is most likely to reduce mutagenesis and cancer risk, and that both research endeavours and dietary recommendations should be redirected away from single molecules to dietary patterns as a main strategy for public health policy.

## 1. Introduction

Strategies for cancer prevention necessarily focus on eliminating unhealthy lifestyle habits such as alcoholism or cigarette smoking or improving both diet and exercise patterns which are believed to contribute to about one-third of annual cancer deaths worldwide [1–4]. Over the last decades, accumulating epidemiological evidence and animal investigations have suggested that consumption of a diet rich in food plants significantly reduces the risk of several types of cancers and recent recommendations point to plant-based diets [5–7]. This raises the theoretical possibility that such protective effects could be attributed to specific micronutrient or phytochemical constituents of food plants and that such components might have beneficial effects in the field of cancer chemoprevention either as naturally occurring dietary constituents/pharmaceuticals or in functional foods [8–10].

It has been speculated that they could manipulate the activity of metabolic enzymes that break down chemical mutagens and carcinogens to reduce lifetime cancer risk. It is

indeed widely believed that the postoxidative enzymes (also, i.e., phase II enzymes), such as glutathione *S*-transferase, UDP-glucuronosyl transferase, sulphotransferase, and acetyl transferase, are able to promote health by detoxifying xenobiotics. On the contrary, the oxidative enzymes (e.g., phase I), represented mainly by the superfamily of cytochrome P450 (CYP) and FAD-containing monooxygenases, raise cancer risk by the bioactivation of ubiquitous mutagenic compounds [11–17]. This rather simplistic dichotomy has in turn suggested that food plant-derived nutrients or phytochemicals might be employed to reduce the risk of cancer through two enzyme-based strategies such as boosting the “good” detoxifying phase II enzymes (using, for example, representative phytochemical-containing fruits and vegetables such as grapes, cauliflower, kale, and broccoli), or inhibiting the “bad” activating phase I enzymes (using those contained in garlic, tea, and onion).

We must remember here that these strategies were extrapolated from epidemiological observations on populations consuming diets varying in both quantity and type of food plant containing thousands of chemical agents which

are able to modulate the specific activity of the metabolizing enzyme battery in a very complex way. They have been popularized by the media and exploited by marketers of supplements of phytochemicals and desiccated vegetables labelled as containing suitable amounts of detoxifying enzyme modulators.

However, this approach totally fails to address the complexity of the multiple interactions between dietary components and xenobiotic metabolism simultaneously generating health benefits or harmful outcomes, depending on circumstances that cannot yet be predicted. Consequently, the potential effects of whole-food plant-derived single constituents on xenobiotic metabolism and cancer risk are also uncertain.

## 2. The Metabolic Manipulation Approach

This modulation strategy foresees large-scale induction of postoxidative phase II enzymes that “detoxify” xenobiotics by means of single green constituents, thereby accelerating the clearance of mutagens and protecting cells against cancer. The potential benefits of this strategy have stimulated active *in vitro* and *ex vivo* studies on the molecular mechanism and specificity of such chemical compounds [18–23]. Particular attention has been devoted to cruciferous vegetables of the Brassica genus, such as kale, cabbage, broccoli, Brussels sprouts, and cauliflower. These vegetables contain considerable amounts of glucosinolates which are the precursors (via the enzymatic conversion by the enzyme myrosinase) of isothiocyanates [24–26], which are phase II enzyme inducers [27–30]. Some researchers have actually created hybrid plants specifically to produce higher amounts of single phytochemical inducers [31]. Resveratrol, a phytoalexin found in grapes and other food products, is also able to boost postoxidative-linked activities [23], but many other compounds contained in plants could be cited.

An alternative anticancer approach is to inhibit the oxidative “bioactivating” phase I enzymes [12, 13]. This hypothesis is emphasized by both the scientific literature and the media, as exemplified by numerous reports urging regular consumption of green or black tea containing catechins as well as onion and garlic rich in diallyl sulfide [32–34].

Finally, both proposed strategies also must be considered in the context of genetic metabolic polymorphisms, which may differentially, *per se*, modulate the effects of any one dietary factors on individuals.

## 3. The Limitations of Such Strategies

We would like to point out that the main difficulty with these strategies is that they totally ignore the complexity of metabolizing enzymatic machinery. Indeed, if on one hand the consumption of food plants, which contain thousands and thousands of phytochemicals (an apple, e.g., seems to contain more than 700 chemical compounds, and a simple fruit salad?) is linked to a reduced cancer risk, on the other, the induction of xenobiotic metabolism by one

specific food component may also stimulate the unwanted formation of highly reactive mutagens [35, 36]. The use of single naturally occurring dietary constituents such as isothiocyanates or individual drugs such as disulfiram, oltipraz, or food additives such as BHA [2(3)-tert-butyl-4-hydroxyanisole], for example, also elicits unhealthy effects [37–40].

It should be pointed out that in addition to the increase in xenobiotic clearance, each postoxidative (phase II) enzyme is also involved in electrophilic species generation and, therefore, must be considered as a “bioactivating system” for specific chemical classes such as halogenated hydrocarbons by glutathione S-transferases, for example, or polycyclic aromatic hydrocarbons (PAHs) by sulphotransferases [41–64]. So, the activation or inactivation of a compound depends on the chemical nature of the compound itself and not on the metabolic enzyme involved. More in general, the manipulation of the activity of one or more phase II enzyme can either increase or reduce the bioactivation of specific compounds. Whereas induction increases the detoxification of some promutagens, thereby favoring chemoprevention, it also increases the bioactivation of countless other foreign chemicals to which humans are simultaneously exposed. As the population is exposed to a myriad of potentially harmful molecules, any modification of the activity of these enzymes could actually lead to unexpected dangerous effects [40]. For example, cruciferous isothiocyanates such as the sulforaphane, widely considered as a beneficial phase II inducer, turn out to be genotoxic or a strong promoter of urinary bladder and liver carcinogenesis, also inducing cell cycle arrest and apoptosis [65–67]. Similarly, engineered *Salmonella typhimurium* TA1535 transfected with the plasmid vector pKK233-2 containing rat glutathione S-transferase 5-5 cDNA has been shown to activate many genotoxins, whereas the nontransfected counterpart does not [68]; in addition, heterologous expression of mammalian theta class glutathione transferases in *S. typhimurium* and *Escherichia coli* systems has been used to demonstrate the role of glutathione conjugation in the genotoxicity of dihalomethanes [61, 69]. Paradoxically, liver metabolic S9 fractions isolated from rodents treated with the monofunctional postoxidative inducer BHA have been proposed as a “complementary” S9 metabolizing system to bioactivate pro-mutagens in typical short-term mutagenicity bioassays [70].

Similar considerations should be made for the inhibitory strategy, a hypothesis that has stimulated recommendations to increase, for example, consumption of green and black teas, as they contain phytochemicals such as catechins able to inhibit the oxidative (phase I) enzymes thus reducing the production of mutagens and carcinogens such as N-nitroso compounds [13, 71]. The inhibition of dimethylhydrazine-induced colon cancer by diallyl sulfide, a flavour component of garlic (*Allium sativum*), has encouraged garlic consumption increase [72, 73]. Moreover, the flavonoid naringin, present in grapefruit and related citrus fruits, has been found to inhibit aflatoxin B1 activation by CYP3A4 in cells and animal models supporting the general idea that green-based metabolism inhibition may reduce carcinogenesis risk [74].

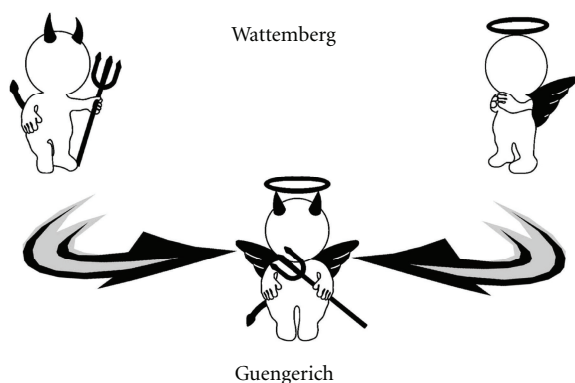


FIGURE 1: Allegoric representation of main difference between classic theory and data emerging from scientific literature. Since 1985 Wattenberg proposed chemoprevention strategies, including the ones that foresee the above mentioned manipulation of metabolic (according to the belief that they are classified as “bad-phase I” and “good-phase II”) enzyme activities, and Guengerich published a comprehensive review that suggested the “dual bioactivating and detoxifying nature” of each metabolic enzyme regardless of whether belongs to the phase I or II battery. The last theory (Guengerich) seems the correct ones, since the data emerging from scientific literature.

The concept of metabolic manipulation, however, ignores the simple fact that the “institutional” role of CYP superfamily toward xenobiotics is to promote their detoxification from the cells and body as well [75]: it is our misfortune that some of them are bioactivated. In other words, due to the dual beneficial and detrimental nature of any of the P450 enzymes, the reduced activation of certain toxins occurs simultaneously with the reduced detoxification of other environmental toxicants to which humans are exposed daily, a phenomenon clearly unpredictable. Once again, it should be noted that the bioactivation of chemicals by phase I apparatus, as occurs for phase II ones, depends on the nature of the substance itself and not on the involved enzyme. Of course, as it is impossible to select safe, personalized human exposure levels to environmental toxins in such a way as to systematically avoid harmful compounds, the regular inhibition of oxidative enzymes might actually lead to an increase toxicological risk. In addition, it should take into account that a selective inhibitor of one CYP enzyme (and due to the existence of many CYP isoforms this strategy should foresee the use a cocktail of multiple inhibitors) may be an inducer of other CYPs; for example, phenethyl isothiocyanate derived from Brassica and diallyl sulfide from garlic are able to inhibit CYP2E1 but also induce CYP2B1 and CYP1A2 [76].

Paradoxically, in 1985 when Wattenberg proposed chemoprevention strategies [12], including the ones that foresee the above mentioned manipulation of metabolic (according to the belief that they are classified as “bad-phase I” and “good-phase II”) enzyme activities, Guengerich published a comprehensive review on the “dual bioactivating and detoxifying nature” of each metabolic enzyme regardless of whether belongs to the phase I or II battery (Figure 1) [41].

Not least, the use of enzyme activity modulators can lead to other serious unhealthy consequences stemming from the alteration of endogenous metabolism where these catalysts are involved (e.g., arachidonic acid derivatives, nitric oxide, aldosterone, cholesterol, or vitamins) as well as alteration of fundamental physiological functions (growth, differentiation, apoptosis, homeostasis, and neuroendocrine functions) [77]; the effects on the pharmacokinetics of coadministered drugs should not be overlooked as well.

#### 4. The Role of Metabolic Polymorphisms

The illogical effects of single daily consumed dietary constituents on xenobiotic metabolism are further complicated by genetic (metabolic) polymorphisms that lead to the occurrence of high- or low-metabolizer phenotypes in the population, each at increased toxicological risk from exposure to specific chemicals [78, 79]. The multiple polymorphisms (e.g., occurrence of high or low (or intermediate in some cases) metabolizers for any oxidative or postoxidative isoforms) characterizing the so called “individual metabolic fingerprint” further complicate the issue. This phenomenon can indeed be interpreted as a sort of a “constitutive up- or down-regulation” of any phase I or II dependent enzyme. In other words, the infinite number of possible combinations of human genetic metabolic polymorphisms constitutes another set of variables in the xenobiotic metabolism [80]. Thus, it appears even more clear that the possibility of manipulating enzyme activity, which in its “constitutive” diversity already may determine genetic disorders as well as perturbations on the chemical biotransformation (including drugs), raises further questions about the effectiveness of the chemical-based enzymatic modulation of cancer risk [81, 82]. In our opinion, these considerations suggest the need for considerable caution before allowing for any form of enzyme-activity manipulation for a generalized prevention, particularly in healthy individuals.

#### 5. On the Clinical Significance

What is the clinical significance of the perpetual manipulation of such enzymatic systems by single nutrient or phytochemicals? Summarizing the various aspects depicted above, the scenario that arises shows how both oxidative and postoxidative enzymes are highly multifunctional and can be induced or inhibited or both by a great number of dietary components. Noteworthy, is the often ignored existence of the dual activating and detoxicating nature of these enzymatic systems. So, the impressive number of chemical compounds that can modulate them, the presence in greens of chemicals that induce both activation and inhibition of mutagenesis, the genetically determined interindividual variability that may moderate (increasing and/or inhibiting) the effects of specific dietary factors on any metabolic enzyme, and the complexity of the interactions among food constituents and enzyme systems have fed the ongoing debates as to whether phytochemicals can alone explain the anticancer ability of plants [9, 83].

It is very difficult to imagine how a single phytochemical, today selected as representative of this or that green, such as lycopene in tomato, resveratrol in grapes, sulforaphane in broccoli, and beta-carotene in carrots, used as a food supplement would offer an advantage, since a variety of fruit and vegetables seems necessary to provide the mixture of vitamins and minerals that appear to favour protection against neoplasia [84]. How can we imagine that the beneficial effects of consuming entire fruits and vegetables, in which enzyme modulating components appear in varying amounts and proportions, and in which unpredictable synergistic and antagonistic (or both depending on the enzyme involved) interactions occur among thousands of different chemicals in their natural matrix, could be just reproduced by supplements of single representative phytochemicals? [85] The beneficial or harmful outcomes of a single compound (*portio facit venenum*, Paracelso) can be quite different from those elicited by the same compound within complex mixtures (*portio and interactiones faciunt venenum*) [86].

The fact that a great number of clinical investigations using single “natural” components failed to reproduce the beneficial effects of the plants from which they were derived should not be underestimated. For example, we can cite the “unexpected” results of cancer chemoprevention trials of antioxidant provitamins and vitamins which we believe can constitute an exemplary warning about the vulnerability of single-nutrient strategies [87–90]. Beta-carotene administered alone or in combination with Vitamins A, E, or C for the prevention of lung cancer and other cancers in heavy smokers or asbestos workers failed to reduce cancer risk and, in some cases, actually increased the risk, raising the suspicion that single chemical supplements may have harmful and beneficial effects as well [91–93]. It has been documented that the deleterious effect of beta carotene can be linked to its ability to stimulate the metabolizing machinery, such as activators of polycyclic aromatic hydrocarbons, and to generate an oxidative stress [94]. In addition, supplementation with commercial doses of vitamin C for 6 weeks is enough to induce DNA damage in human lymphocytes [95], probably by means of its ability to generate oxidative stress connected to phase I upregulation [96, 97].

## 6. Concluding Remarks

In the field of cancer prevention, the idea of producing the so called “magic-bullet,” as conceived by Paul Ehlich for antibacterials, too easily evokes the long-life elixir on a molecular level capturing the imagination of both the public and researchers. From the standpoint of cancer research policy, the possible role of single dietary constituents is of pivotal interest in cancer research but basic information about the role of metabolizing apparatus, however, makes it clear that the role of any single anticarcinogenic phytochemical cannot be understood except in the context of broader dietary patterns. The ongoing scientific controversy surrounding the effects of single molecules on cancer risk seems to provide a salutary warning for health policymakers. Considering that unhealthy lifestyle factors are also taken into account,

educational campaigns encouraging the consumption of fruit, fibres, and greens should be encouraged.

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