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The Role of Crucifers in Cancer Chemoprotection

Jed W. Fahey and Paul Talalay

Department of Pharmacology and Molecular Sciences, Brassica Chemoprotection Laboratory / 406 WBSB, The Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205-2185

INTRODUCTION

The magnitude of the cancer problem is staggering: over a million new cases are diagnosed and one-half million deaths occur each year in the United States alone. Despite the great advances in biomedical sciences over the past 20 years, we have made very little progress in preventing death due to cancer. In the past 20 years, age-adjusted cancer mortality and incidence (all organ sites excluding lung cancer, male and female) has actually increased, and five-year survival rates have improved only marginally in that time period. Although worldwide the age-adjusted cancer rate doesn't vary greatly, rates of organ-specific cancers vary by as much as 300-fold, and diet probably plays a major role in these differences (4).

Persuasive epidemiological evidence underscores the fact that the majority, perhaps 75%, of human cancers are related to extrinsic factors such as smoking, diet, alcohol consumption, radiation, infection, drugs, pollutants, and even sexual and reproductive practices. Smoking and diet are generally acknowledged to be the most important of these extrinsic factors (3,4). Dietary major (nutrient and non-nutrient [e.g. fiber]) and minor (e.g. additive, contaminant, and plant secondary product) components can both reduce and enhance cancer incidence and progression. Anticancer properties have been reported for many phytochemicals, including β-carotene, tannins, coumarins, flavonoids, phenols, terpenes, and isothiocyanates (15).

High vegetable and fruit consumption is associated with a striking reduction in susceptibility to malignancy. The mechanisms underlying this phenomenon are complex and incompletely understood. The contribution of minor plant components to this protection against malignancy has received relatively little attention. Experimental studies have shown that chemical protection against carcinogenesis, mutagenesis, and other forms of electrophile toxicity can be achieved by raising the activities of enzymes involved in xenobiotic metabolism, particularly Phase

2 enzymes such as glutathione transferases, glucuronosyltransferases, and quinone reductase. Edible plants, particularly crucifers, contain numerous minor chemical components that elevate Phase 2 enzymes and also protect against carcinogenesis.

Both the epidemiology and the mechanisms of the chemoprotective effect afforded by increased fruit and vegetable consumption were reviewed by Steinmetz and Potter (10, 11). Block et al. (2) conclude that "major public health benefits could be achieved by substantially increasing consumption of these foods" (fruits and vegetables). In their recent exhaustive review of dietary studies relating cancer risk to quantity of fruit and vegetables consumed, they showed that with increased consumption there was a strong and consistent reduction in relative risk for many types of cancer in 128 of 156 studies analyzed. It is far from clear to what extent the nutrient or non-nutrient components of these foods may reduce risk or whether an indirect effect (e.g. a parallel reduction in fat intake) is primarily responsible for this protection. There are numerous studies showing chemoprotective activity of individual chemical constituents of vegetables, however, the availability of which offers the opportunity to study chemoprotection by pure dietary components. The health benefits to be realized by increased consumption of the vegetable *Brassica* species have been cited frequently, and were most recently reviewed by Beecher (1). We may therefore conclude that much of human cancer is avoidable by a combination of prevention (the elimination of exposure to carcinogens or carcinogenic practices) and chemoprotection (chemical or dietary interventions designed to reduce susceptibility to carcinogens by blocking, retarding, or reversing susceptibility to cancers).

CARCINOGENESIS

The process of carcinogenesis consists of a number of phases. During the first or initiation phase, genetic changes (DNA damage) occur. Many chemical carcinogens are highly reactive, electrophilic (positively-charged) chemical species that can initiate carcinogenesis by reacting spontaneously and damaging negatively-charged phosphate linkages of DNA bases. Following this initiation phase there may be a subsequent quiescent phase, followed by multiple steps commonly referred to as promotion, conversion or expression, and progression during which altered or damaged DNA in the initiated cell is expressed, leading to a preneoplastic lesion through selective clonal expansion and ultimately leading to neoplasia and the clinical manifestation of cancer. Carcinogenesis, however, can be interrupted during the early or initiation phase, by a variety of chemical agents, many of which are already in the human diet (15).

CHEMOPROTECTION

Many so-called carcinogens that enter the body are actually quite innocuous procarcinogens that are activated by Phase 1 enzymes, only then becoming true carcinogens. The effect of these Phase 1 enzymes (e.g. certain cytochromes P450)

is counterbalanced by a family of enzymes known as Phase 2 enzymes (e.g. glutathione transferases, quinone reductase, glucuronosyltransferases, and epoxide hydrolases). These enzymes conjugate reactive carcinogens with glutathione, glucuronic acid, or other tissue constituents, thereby detoxifying them and facilitating their excretion from the body. A shift in the balance between Phase 1 and Phase 2 enzymes dictates in part whether malignancy will ensue when a carcinogen enters the cell (Fig. 1). Since the likelihood of cancer increases when the Phase 2 detoxification mechanism is overwhelmed, it has been proposed that carcinogenesis may be blocked by enhancing Phase 2 enzyme activity, thus shifting the balance of these enzymatic reactions towards detoxication (12). The activities of both the Phase 1 and Phase 2 families of enzymes that metabolize xenobiotics, including carcinogens, are elevated or induced by low concentrations of a wide variety of chemical substances. Wattenberg (15) demonstrated in the early 1970s that butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are widely added to processed food as protective antioxidants, could block the formation of tumors in several rodent organs (eg. forestomach, lung, and mammary gland) elicited by many quite different types of chemical agents. These important observations showed that compounds already widely consumed in the human diet, albeit as additives, were chemoprotectors. Extensive analysis of the molecular mechanisms of the chemoprotective activities of BHA and BHT led to several important conclusions and the identification of a novel strategy for achieving chemoprotection (13).

Inducers of Phase 1 and Phase 2 enzymes of xenobiotic metabolism are of two distinct types: monofunctional inducers, including BHA and BHT, which induce Phase 2 enzymes selectively without a significant effect on Phase 1 enzymes, and bifunctional inducers, including many carcinogenic compounds, which induce both Phase 1 and Phase 2 enzymes (7). All monofunctional inducers of Phase 2 enzymes that have been examined thus far have shown tumor-blocking activity in animals. Such inducers include phenolic antioxidants (e.g. BHA and BHT),

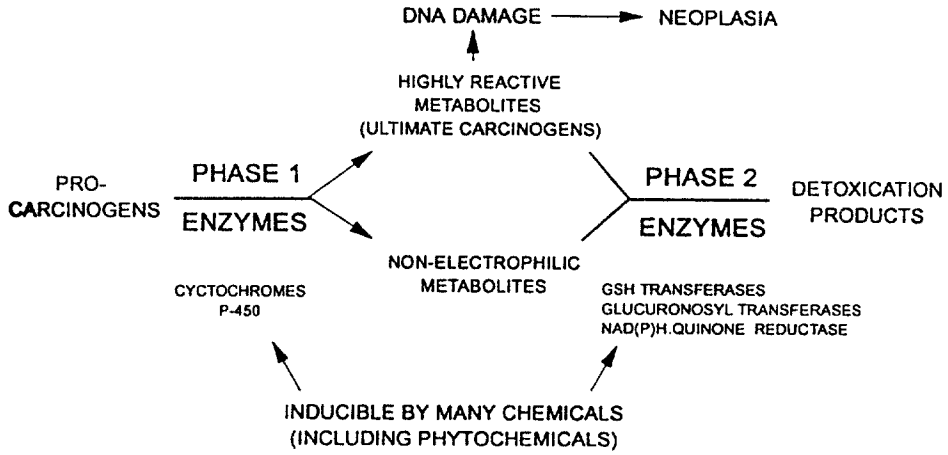

Figure 1. General pattern of carcinogen metabolism. Targets for chemoprotection.

organic isothiocyanates, dithiocarbamates, fumarates, coumarins, cinnamates, and 1,2-dithiole-3-thiones (12).

EXPERIMENTAL SYSTEM

In light of the compelling evidence that substances that elevate Phase 2 xenobiotic metabolizing enzymes also reduce the susceptibility of animals to the toxic and neoplastic effects of carcinogens, simple experimental systems for identifying novel monofunctional inducers, measuring their potencies, and elucidating the molecular events responsible for induction were developed. A particularly valuable and easily measured marker enzyme suitable for these purposes is a nicotinamide nucleotide-dependent quinone reductase that is widely distributed in many cells and tissues, and that protects cells against the toxicities of quinones which are present in many plants (8). Measurement of the specific activities of quinone reductase in cells of a murine hepatoma cell line (Hepa 1c1c7) provides rapid information on the potencies and toxicities of inducers (5, 6).

INDUCTION OF PHASE 2 ENZYMES BY DIETARY COMPONENTS

Backed by the conclusion that substances which induce Phase 2 enzymes are highly likely to be anticarcinogens and by the availability of a cell culture system for the simple and rapid detection of such activity, a survey of organic solvent extracts of commonly-consumed vegetables for such inducer activity was undertaken by Prochaska et al. (6). This survey pointed to the Cruciferae, particularly *Brassica* species, as singularly rich sources of inducer activity (Fig. 2). It also revealed that these *Brassica* species varied considerably in their inducer activity,

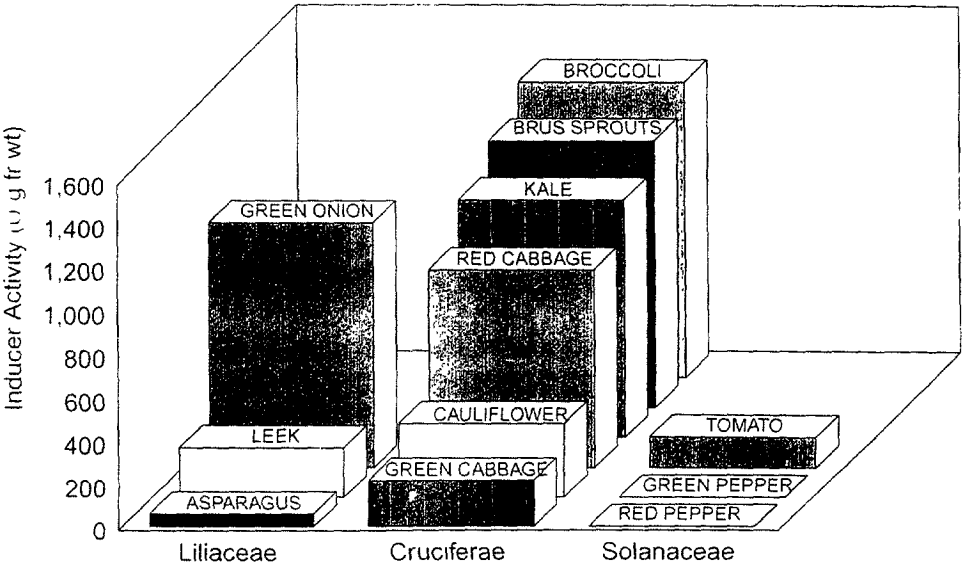

Figure 2. Potency of quinone reductase induction by acetonitrile extracts from three families of commonly consumed vegetables.

and that in addition to the genetic influence (eg. species, variety, cultivar), growth conditions and time of harvest were also likely to govern the magnitude of the inducer activity.

BROCCOLI: THE PROTOTYPICAL CRUCIFEROUS VEGETABLE

Since broccoli was determined to be especially rich in inducer activity in the survey described above, broccoli cultivars were selected for further study. The fact that broccoli is consumed in large amounts in the United States and that its consumption increased eightfold in the last 20 years also makes this an attractive vegetable for potential development as a nutraceutical. Chromatographic fractionation of organic solvent extracts of a lyophilized, cold-water homogenate of organically grown broccoli heads demonstrated that most of the inducer activity was confined to a single fraction, which upon further purification yielded a single compound (18). Spectroscopic identification and confirmation by synthesis showed the inducer to be an isothiocyanate, presumably largely formed by the action of myrosinase on glucoraphanin during the initial processing. This compound, sulforaphane, has the structure CH_3-S(O)-$(CH_2)_4$-NCS (18), and has been isolated previously from a Cruciferous weed: hoary cress, *Cardaria draba* (9). Sulforaphane was found to be an extremely potent inducer of quinone reductase in murine hepatoma cells, and was far more potent than any previously identified naturally-occurring inducer. It induced both quinone reductase and glutathione transferases in a variety of mouse tissues, and was shown to be able to block 9,10-dimethyl-1,2-benzanthracene-induced mammary carcinogenesis in female Sprague-Dawley rats (16). The finding that the majority of the inducer activity of extracts of broccoli cv SAGA resides in a single chemical entity, an isothiocyanate or mustard oil presumably derived from a glucosinolate, is of considerable interest. Glucosinolates such as glucoraphanin are abundantly distributed in the Cruciferae, which when wounded or chewed release the compartmentally segregated enzyme myrosinase, leading to conversion of the glucosinolates primarily to their isothiocyanate aglycones (Fig. 3). All of the naturally-occurring organic isothiocyanates examined in our laboratory induce the enzymes of xenobiotic metabolism, and many have been demonstrated to be inhibitors of chemical carcinogenesis in several models (see review by Zhang and Talalay, 17). Further

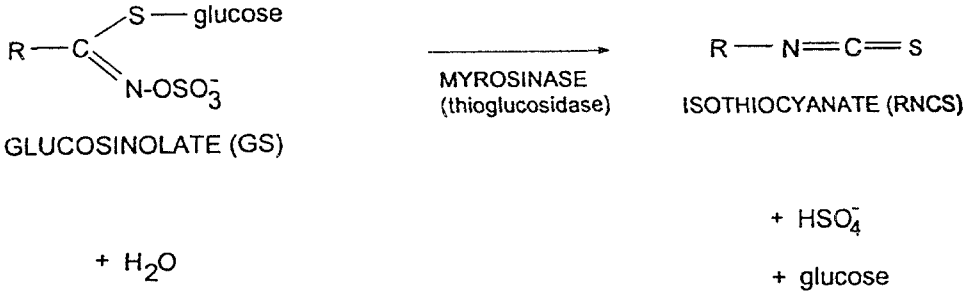

Figure 3. Conversion of glucosinolates to isothiocyanates.

examination of broccoli and other crucifers grown under controlled environmental conditions has confirmed the earlier findings of Prochaska et al. (6) that broccoli (N=8 cultivars) is a more potent inducer of Phase 2 enzymes than other Cruciferae, including arugula, red cabbage, white cabbage, Chinese cabbage, peppergrass, curly cress, daikon, GyLon, mustard greens, Brussels sprouts, kale, collards, red radish, oilseed mustard, and watercress (unpublished data).

CONCLUSION

Our efforts to understand the many complex effects of diet on cancer incidence have focused on minor dietary constituents that raise the activities of cellular enzymes affecting the metabolic detoxication of carcinogens. Much evidence indicates that induction of these enzymes blocks the formation of tumors in experimental animals. To detect and quantitate inducer activity, a simple and rapid cell culture system that depends on measuring one Phase 2 enzyme was devised in our laboratory. This system identified vegetables rich in inducer activity, and led to the isolation from broccoli of sulforaphane (an isothiocyanate or mustard oil) that is a very potent enzyme inducer and anticarcinogen. A comprehensive evaluation of edible Cruciferae for their ability to boost protective enzymes appears to be a promising approach toward devising dietary strategies for reducing the risk of cancer.

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