Inhibition of Carcinogenesis by Minor Dietary Constituents

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Abstract

Cancer can be prevented by a surprisingly large number of chemical compounds. This type of prevention is referred to as chemoprevention. Two forms exist: one, general population chemoprevention, entails efforts at providing measures applicable to large population groups; the other, targeted chemoprevention, is directed toward individuals at increased risk of cancer in specific tissues. The two differ in terms of allowable toxicity and specificity of agent targeting. Driving forces for general population chemoprevention have been both epidemiological and experimental data indicating protective effects of dietary constituents against the occurrence of cancer. Epidemiological studies have shown that diets containing large quantities of vegetables and, to a lesser extent, fruits are associated with relatively low risks of cancer. The assignment of which constituents of these foods of plant origin are responsible for protection has been indecisive. In the search for inhibitory compounds, a group currently under investigation is that of the minor nonnutrient constituents of the diet. This presentation provides an overview of experimental studies in which members of this very diverse group of compounds have been shown to inhibit carcinogenesis. The implications of these data could be quite profound. Ultimately, they could be relevant to decisions on food selection and composition for the purpose of providing protection against neoplasia.

Comparisons between the Effects of Crude and Semipurified Diets on the Carcinogenic Response of Experimental Animals

Studies from the early work of Silverstone et al. (1) demonstrated that in some experiments animals fed a crude diet were at lesser risk of developing cancer than were those fed a semipurified diet. The crude diets (Purina chow) contain components such as grain, beet pulp, alfalfa meal, cane molasses, fish meal, and other crude materials. In contrast, semipurified diets consist of defined dietary constituents, such as casein, starch, corn oil, minerals, and vitamins. The term semipurified means that some of these constituents, principally vegetable oils, are not pure. The two types of diets are nutritionally comparable but differ profoundly in amounts of nonnutrients. In one investigation, male DBA or C3H mice placed on the two different types of diets developed hepatomas spontaneously. In a series of experiments, 2 to 5 times as many of the mice fed the semipurified diet developed hepatomas compared with groups fed the crude diet. In some experiments of cancers of other sites such as skin cancers, mammary cancers, and leukemias, the results were indecisive or no effects were found.

More recently, the effects of feeding AIN-76, a semipurified diet, and NIH-07, a crude diet, were compared for carcinogen-induced neoplasia in mice given the tobacco-specific carcinogen, NNK (2). Feeding of the diats was begun 2 weeks before carcinogen challenge and continued for the duration of the protocol. The animals fed the semipurified diet had 3 times as many pulmonary adenomas as did those fed the crude diet.

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The abbreviations used are: NNK, 4-(methyltritosamino)-1,3-(pyridyl)-1-butanone; BP, benzo(a)pyrene; DMBA, 7,12-dimethylbenz(a)anthracene; AMT, allyl methyl trisulfide; AMD, allyl methyl disulfide; DAT, diallyl trisulfide; DAS, diallyl sulfide.
These data agree with epidemiological investigations in which a protection by vegetables against lung cancer in the humans was observed (5).

In other experiments, the comparisons focused on either the initiation or postinitiation phases of carcinogenesis. One study compared a crude diet (Purina rat chow) with a semipurified diet (Teklad) in mice during the initiation phase. Two experiments were performed, one with p-propiolactone and one with BP (3). Animals were placed on the experimental diets 2 weeks before carcinogen challenge and were maintained on the diets until 1 day after the last dose of carcinogen. The target organ was the forestomach. In the first experiment, mice treated with p-propiolactone and fed the crude diet had approximately one-third as many forestomach tumors as did those fed the semipurified diet. In the second experiment, with BP-induced neoplasia, no difference in carcinogenic response was observed. This experiment shows that the dietary effects on initiation by different carcinogens can vary.

A study aimed at investigating the effects of feeding a crude with a semipurified diet on the postinitiation phase of carcinogenesis was carried out by Longnecker et al. (4, 6). The experimental model was azaserine-induced neoplasia of the pancreas in rats. These investigators showed that feeding a crude diet during the postinitiation phase resulted in a lower incidence of pancreatic neoplasia compared with feeding a semipurified diet. In summary, a number of experiments show that the carcinogenic response of animals fed crude diets was less than in those fed a semipurified diet. The crude diets contain an abundance of nonnutrient dietary constituents whereas the semipurified diets contain relatively small quantities. Thus, these studies provide an initial indication that nonnutrient dietary constituents are inhibitors of carcinogenesis.

Effects of Single Crude Dietary Constituents on Carcinogen-induced Neoplasia in Experimental Animals

Single crude dietary constituents have been added to semipurified diets so that their effects on the carcinogenic response could be studied. The results of adding cruciferous vegetables were studied with DMBA-induced neoplasia in Sprague-Dawley rats. In this experimental model, the animals develop mammary tumors after a single administration of DMBA by p.o. intubation. Frozen-thawed pieces of vegetable were placed in the cages once a day (7). The animals consume this type of vegetable supplement very rapidly thereby mimicking human meal consumption. The vegetable supplements were provided beginning 1 week after carcinogen administration only during the postinitiation phase of carcinogenesis. Under these conditions, cabbage and broccoli inhibited mammary tumor formation. In another study with this tumor model, orange oil was added to a semipurified diet. A dose-dependent inhibition of mammary tumor formation resulted (8). In other experiments, the effects of onion and garlic oils on epidermal carcinogenesis in mouse were studied. Topical applications of these two oils produced inhibition in the postinitiation phase in this animal model (9). In other work, extracts of plant materials containing protease inhibitors inhibited the postinitiation phase of carcinogenesis of the skin, breast, large bowel, and lung (10). Green tea administered in the drinking water was recently shown to inhibit carcinogenesis in mice (11).4

4 A. Conney, unpublished observations.

Inhibition of Carcinogenesis by Specific Nonnutrient Constituents of the Diet

The finding of specific compounds of plant origin with inhibitory effects on carcinogenesis has resulted from two lines of investigation. One of these is the identification of a constituent in crude plant materials. The plant material used had carcinogen-inhibitory effects or produced a biological response, such as enhancement of detoxification systems, likely to result in cancer prevention. In these studies, d-limonene, a major constituent of citrus fruit oils that inhibits carcinogenesis, was found to be preventive (12). Several groups of inhibitors also were identified in cruciferous vegetables. These include indoles, aromatic isothiocyanates, dithiolethiones, and phenols. Studies to identify the active constituents of garlic and onion oils that inhibit carcinogenesis showed a number of organosulfur compounds in these oils that have inhibitory properties (13, 14).

The second type of investigation that identified nonnutrient dietary inhibitors focused initially on synthetic compounds. Subsequently, these structurally related compounds were found in natural products. Initial studies of synthetic chemicals resulted in identification of flavone as an inhibitor. Flavone, itself, is a synthetic compound. However, a large number of naturally occurring flavone derivatives were found to inhibit carcinogenesis (15–17).

The mechanisms of action of many of the minor nonnutrient inhibitors of carcinogenesis are poorly understood, making it difficult to organize them into a precise pattern. One organizational framework is classification of inhibitors by the time in carcinogenesis when they are effective. In this framework, inhibitors can be divided into three categories. The first consists of compounds that prevent the formation of carcinogens from precursor substances. The second contains compounds that inhibit carcinogenesis by preventing carcinogenic agents from reaching or reacting with critical target sites in the tissues. These inhibitors are called blocking agents, which describes their mechanism of action (they exert a barrier function). Inhibitors of the third category act subsequent to exposures to carcinogenic agents. These are termed suppressing agents because they act by suppressing the expression of neoplasia in cells previously exposed to doses of carcinogens that otherwise would cause cancer (15). Studies of various inhibitors have made it apparent that some inhibitors act at more than one time in the carcinogenic process (7).

Blocking Agents

Blocking agents prevent carcinogens from reaching or reacting with critical target sites by several mechanisms. One mechanism is inhibiting of reactions requiring metabolic activation. A second entails inducing of activities of enzyme systems that detoxify carcinogens. A third is the trapping of reactive carcinogenic species. Some blocking agents prevent the action of tumor promoters by preventing the promoter from reaching or reacting with its cellular target or by blocking subsequent cellular events required for tumor promotion to occur. A list of nonnutrient blocking agents is shown in Table 1.

Blocking Agents That Act by Inhibiting Carcinogen Activation. Several groups of nonnutrient compounds prevent carcinogenesis by inhibiting carcinogen activation reactions (Table 2). Almost all carcinogens occurring in food require metabolic activation. Examples of such carcinogens are include aflatoxin, nitrosamines, polycyclic aromatic hydrocarbons, hydrazines,
and heterocyclic amines. Thus, blocking agents inhibiting activation of these and other carcinogens may play a role in preventing neoplasia resulting from this type of exposure. Benzyl isothiocyanate is one such naturally occurring blocking agent. Benzyl isothiocyanate, found in cruciferous vegetables, was found to inhibit the occurrence of pulmonary adenomas in these animals. More recently, D-limonene and citrus fruit oils were found to inhibit the carcinogenicity of NNK (48).

Glucobrassicin and glucotropaeolin, two glucosinolates occurring in cruciferous vegetables, were also found to inhibit DMBA-induced mammary neoplasia in rats when administered by gavage 4 h before the carcinogen. Thus, data now show that four widely occurring groups of naturally occurring compounds, i.e., aromatic isothiocyanates, organosulfur compounds found in Allium species, monoterpenes, and glucosinolates, can inhibit carcinogenesis if administered shortly before carcinogen challenge (Table 2).

Blocking Agents That Act by Increasing Carcinogen Detoxification. Blocking agents can prevent the occurrence of neoplasia by increasing the detoxification of carcinogens. Two general categories of blocking agents acting by this mechanism have been identified. They are designated type A and type B inhibitors (15). Type A inhibitors induce an increase in activity of phase 2 enzymes, which perform conjugation and other reactions that detoxify many carcinogenic agents. Prominent among these is the marked increase in glutathione S-transferase activity. UDP-glucuronosyltransferase, epoxide hydrolase, and NAD(P)H-quinone reductase activities are also enhanced. Increases in tissue glutathione levels are found. Type B inhibitors enhance both phase 1 and phase 2 enzyme activities. The phase 1 enzymes include the cytochrome P-450 systems and are very complex. Reviews of the effects of type A and type B inhibitors have been published (15).

Because type A inhibitors are effective, there have been a continuing effort to identify compounds in this category of chemopreventive agents. Recent work has focused on the organosulfur compounds found in Allium species. In initial studies, the capacity of AMT to induce increased glutathione S-transferase activity in rodent tissues was investigated. AMT induces increased activity of this enzyme system when given 4 and 2 days before assay. With this administration schedule, AMT was also found to inhibit BP-induced neoplasia of the forestomach of female A/J mice (20). Experimental protocols with the test compound being given 4 and 2 days before carcinogen challenge are commonly used in studying the inhibitory effects of agents that act by inducing increased activity of detoxification systems.

Other organosulfur compounds were studied under these same conditions. Ultimately, the experiments have included four allyl group–containing compounds with one, two, or three linearly connected sulfur atoms: AMT, AMD, DAT, and DAS. Four corresponding saturated compounds in which propyl groups are substituted for the allyl groups also were studied. All four allylic compounds inhibited BP-induced neoplasia of the forestomach. Their saturated analogues showed almost no inhibitory activity, indicating the importance of allyl groups. DAT, with two allyl groups, was more potent than AMT, with one, providing evidence for the role of allyl groups in the inhibitory effects observed. DAS and AMD, but not DAT or AMT, inhibited pulmonary adenoma formation (20). The fact that monosulfide and disulfide inhibit tumor formation in the lung but that trisulfide does not indicates that the number of sulfur atoms in the molecule can control the organ sites at which protection against carcinogenesis occurs. All four allylic compounds induce increased glutathione S-transferase activity in the forestomach but vary in capacity to induce glutathione S-transferase activity in the lung, liver, and small bowel. Their...
saturated analogues produced little or no induction (20). Like the organosulfur compounds from Allium species, aromatic isothiocyanates from cruciferous vegetables, in particular, benzyl isothiocyanate, induce increased glutathione S-transferase activity and have other properties of type A inhibitors.

Blocking Agents Effective against Tumor Promoters. A number of naturally occurring nonnutrient inhibitors of tumor promotion have been found. A property for identifying inhibitors of this type is their capacity to prevent an increased ornithine decarboxylase activity after exposure to the promoter. This assay has been particularly effective in mouse epidermis. However, in other instances, biological responses such as inhibition of increased cellular proliferation or tumor formation in response to promoter administration has been the means by which the inhibitor was identified. Table 1 lists compounds that inhibit tumor promotion, including phenols, flavones, tannins, curcumin, glycyrrhetinic acid, and glucarates. Mechanisms by which these inhibitors work include inhibition of components of the arachidonic acid cascade, antioxidant activity, and modulation of hormonal responses.

Suppressing Agents

Suppressing agents prevent the evolution of the neoplastic process in cells previously exposed to doses of carcinogenic agents that otherwise would cause cancer (15). Table 3 lists minor nonnutrient constituents of the diet that can act as suppressing agents in animal models. The number of suppressing agents identified is considerably smaller than the number of blocking agents. The mechanisms by which blocking agents act are relatively simple, although the systems themselves can be very complex. The mechanism of action for suppressing agents is not well defined, as might be expected because the events that they counteract are those basic to cancer, also a poorly understood process. Suppressing agents act during the earliest stages of the neoplastic process, when the number of pathological alterations is considerably smaller than at later stages (55). As more information becomes available concerning the multiple events leading to malignancy, targeting of suppressing agents to counteract defined early changes may be feasible. The vast amount of data indicates that alterations in genetic material occur during carcinogenesis. Correcting genetic alterations during neoplastic changes in solid tissues is difficult. Suppressing agents can modulate consequences of the genetic changes but are not likely to correct the genetic defects themselves. Accordingly, it would be anticipated that suppressing agents may have reversible effects. Consequently, it is likely that continuous administration of suppressing agents will be necessary to sustain inhibition of carcinogenesis. Retinoids, the most extensively investigated of the suppressing agents, demonstrate this characteristic both in vitro and in vivo. Suppression is maintained as long as the agent is present; on removal, the neoplastic process recurs.

Studies of crude diets for suppressing effects are relatively sparse. One such study cited previously was carried out by Longnecker et al. (4, 6), who demonstrated a suppressing effect of crude diets on the occurrence of pancreatic cancer. This study is not definitive, because animals fed the crude diet gained less weight than did those fed the semipurified diet. It is well established that retarding weight gain also can diminish the carcinogenic response. However, the difference in the incidence of pancreatic neoplasms between the two groups of animals was greater than expected from the modest difference in weight gain.

Suppressing agents have been found in individual dietary constituents. Studies with cruciferous vegetables and with orange oil demonstrated suppressing effects (8). In the initial studies with cruciferous vegetables, dehydrated powders prepared from Chieftain Savoy cabbage inhibited DMBA-induced mammary-tumor formation. In further investigations, it was found that frozen-thawed segments of cabbage leaf placed directly in cages produced a comparable reduction of neoplasia in the animals (56). The amount of cabbage leaf fed was of the order of 5 g/kg body weight/day. More recently, similar studies with frozen-thawed segments of broccoli and cabbage were given beginning 1 week after DMBA and continued for 18 weeks after DMBA. The rats were fed a semipurified diet, and the vegetable additions were given 6 days a week. The results showed that fewer animals receiving cabbage had mammary tumors compared with nonsupplemented controls (100% versus 56%) and that the average number of tumors per rat was diminished markedly (2.5 versus 0.8). Similar findings were obtained with broccoli (56).

Possibly related to the suppressive effects of cruciferous vegetables is the finding that benzyl isothiocyanate, a constituent of these plants, will inhibit DMBA-induced mammary carcinogenesis when administered beginning 1 week after DMBA challenge (15, 38). Suppressive effects also were pro-
duced by orange oil or its principal constituent, d-limonene (8, 12). In recent studies, nerolidol, a sesquiterpene structurally related to farnesol, suppressed azoxymethane-induced neoplasia of the large bowel in rats (57). The observation that suppressing agents occur in commonly consumed foods indicates that further searches should be made for this category of chemopreventive agents.

Table 3 lists some defined minor nonnutrient dietary constituents that have suppressing activity. Included are protease inhibitors, a very interesting group of suppressing agents. The in vitro data showing their suppressive effect are striking. Some in vivo studies have also shown inhibition, but not many of such investigations have been performed (10). Another group of suppressing agents with a common characteristic are inhibitors of components of the arachidonic acid cascade. This group includes various polyphenolic compounds. Studies with synthetic inhibitors of the arachidonic acid cascade, such as indo-

methacin and piroxicam, demonstrated a suppressive effect, thus suggesting that the naturally occurring compounds may act similarly (49–51). Inhibitors of the arachidonic acid cascade can be potent inhibitors of tumor promotion (5). The relationship between their mechanisms of suppression and inhibition of tumor promotion is not clear. Two additional groups of naturally occurring suppressing agents are the terpenes and isothiocyanates (12, 15). Further work is required to more fully evaluate their potential role as suppressing agents.

Most of the research with suppressing agents has focused on synthetic compounds and a few minor dietary compounds that are nutrients. As mentioned previously, the retinoids, synthetic analogues of vitamin A, are the most extensively studied suppressing agents. Vitamin A has only limited suppressing activity (54). It is relatively toxic at the large amounts generally used for suppression. However, in a careful study by Zile et al. (58), moderate supplementation of the diet with vitamin A inhibited carcinogen-induced mammary neoplasia in rats. β-Carotene has also been studied extensively for its suppressing activity in animal systems. Many of these studies have given negative results. A few studies giving positive results were published (59). Selenium salts and inositol hexaphosphate were also shown to have suppressing effects (60–63).

### Inhibitors with Both Blocking and Suppressing Activity

During studies of inhibitors of carcinogenesis, some substances showed both blocking and suppressing effects. The first was sodium cyanate, where both blocking and suppressing effects against DMBA-induced mammary carcinogenesis in rats (15). Subsequently, cruciferous vegetables were shown to have these characteristics, as was one of their constituents, benzyl isothiocyanate. In experiments with cabbage, consumption of a semipurified diet with added dehydrated powders prepared from Chieftain Savoy cabbages showed blocking effects against DMBA-induced mammary carcinogenesis in rats. It was also found that consumption of the cabbage powders beginning 1 week after DMBA administration suppressed mammary tumor formation. Subsequently, benzyl isothiocyanate was shown to block DMBA-induced mammary carcinogenesis and to suppress formation of mammary tumors under similar conditions to those used with crude plant materials (15).

Citrus fruit oils were also found to have both blocking and suppressing capacities. The most extensively studied is orange oil, which has a blocking effect against DMBA-induced mammary neoplasia and a suppressive effect in this experimental model (8). D-Limonene, found in orange oil, was shown to have both blocking and suppressing effects against DMBA-induced mammary tumor formation (12). Inhibitors having both blocking and suppressing capacities, such as cruciferous vegetable powders, benzyl isothiocyanate, orange oil, and d-limonene, have another property in common; They all induce increased glutathione S-transferase activity in mouse tissues. The significance of this relationship remains to be explained.

The finding of inhibitors with both blocking and suppressing activity appears important. However, mechanisms for these dual inhibitory actions are not known. Two distinctive possibilities merit consideration. One is that a coordinated protective system against potentially toxic compounds exists that includes both a detoxification component and a suppressive component. The latter would be a fail-safe means of protection should the former be not completely effective. On exposure to toxic compounds, the detoxification system constitutes the first line of defense. Phase 2 enzymes, as well as glutathione, are components of this detoxification (blocking) defense. Because blocking effects may not always be completely successful, a second line of defense might prevent manifestations of damage that occurs, i.e., suppression.

Whereas a coordinated protective mechanism entailing increased activity of detoxification systems and suppression of manifestations of toxicity appears to be a reasonable possibility, the inclusion of a decrease in cytochrome P-450 enzyme activities might appear incongruous. However, an insight into how such enzyme inhibitions might fit into an overall protective system was provided by experiments that show that some compounds that reduce cytochrome P-450 activity increase interferon levels in tissues (64, 65). Such findings suggest that a decrease in activity of one mechanism that is involved in protection can be accompanied by an enhanced activity of a different protective system.

A recent publication by Nebert (66) conceptualize how blocking and suppressing activity might be brought about by the same compound. This investigator discusses a possible relationship between components of the nuclear receptor superfamily, with foreign chemicals as ligands, and cell proliferation. The mouse peroxizome proliferator-activated receptor, a novel member of the nuclear receptor superfamily, has foreign chemicals as its only ligands (67). Numerous studies show that peroxizome proliferation is associated with liver carcinogenesis. Relationships are proposed between the level of activity of drug-metabolizing (foreign body-metabolizing) enzymes, the steady-state level of small oxygenated molecules (including steroids) that act as signals for growth, differentiation, and possibly tumor promotion. If such interactions exist, opposing systems might exist as well. One would entail inhibition of the activity of the drug-metabolizing systems and a decrease in signals for cell proliferation. Although these conjectures are highly speculative, they form the conceptual basis for relating the activity of systems that detoxify chemical carcinogens to nuclear control mechanisms of cell proliferation. If a defense mechanism with both blocking and suppressing components exists, it could be triggered by a receptor mechanism.

A second possibility that might account for a compound having both blocking and suppressing activity is high reactivity with multiple effects due to this attribute. In such cases, blocking and suppressing effects would be triggered separately. Aromatic isothiocyanates are highly reactive compounds and their capacity to act as both blocking and suppressing agents might be due to their high chemical reactivity. In contrast, d-limonene

20896
is not a highly reactive molecule. However, it is metabolized to epoxides that are highly reactive. The paucity of data makes it difficult to evaluate the two major mechanisms of action considered. If a receptor activating both blocking and suppressing action were identified, it would be a major achievement. Even if such a unified defense system does not exist, knowledge about the characteristics of inhibitors having both blocking and suppressing effects might enhance the possibility of obtaining chemopreventive agents with maximum protective capacities.

Summary

Epidemiological studies have shown that diets containing large quantities of vegetables and, to a lesser extent, fruits are associated with relatively low risks from cancer. The information about which constituents of these foods are responsible for protection has been indecisive. One group of inhibitory compounds currently under investigation consists of minor nonnutrients in diets. Foods of plant origin frequently contain amounts of these substances as high as several per cent. In recent years there has been a growing awareness that these nonnutrient compounds may have important effects on the consequences of exposure to carcinogenic agents. This information comes from studies of whole diets; studies of individual dietary constituents, particularly those of plant origin; and a large number of investigations with pure compounds. The nonnutrient inhibitors of carcinogenesis have several different mechanisms of action. Some are blocking agents; i.e., they prevent carcinogens from reaching or reacting with critical target sites. Others are suppressing agents; i.e., they prevent evolution of the neoplastic process in cells which otherwise would become malignant. Occasionally the same compound will show both mechanisms. As studies of the inhibitory capacities of nonnutrient dietary inhibitors of carcinogenesis continue, a clearer picture of their potential impact in cancer prevention will emerge. Pending definitive data and as a practical consequence of the current state of knowledge, the basic recommendation of the National Research Council, the National Cancer Institute, and the American Cancer Society that the daily diet should contain generous amounts of vegetables and fruits should be followed. For individuals of sufficient socioeconomic status, following this recommendation is a matter of education and choice. For the economically deprived, making available diets that decrease the risk of cancer involves a public health concern.

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