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Potential of Food Modification in Cancer Prevention[1]

Clement Ip,[2] Donald J. Lisk, and Joseph A. Scimeca

Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, New York 14263 [C. I.]; Department of Fruit and Vegetable Science, Cornell University, Ithaca, New York 14853 [D. J. L.]; and Nutrition Department, Kraft General Foods, Glenview, Illinois 60025 [J. A. S.]

Abstract

This presentation focuses on research that could theoretically be applied to implement the strategy of general population chemoprevention. The concept is based on the premise of enhancing foods with known anticarcinogens through either agricultural methods or food-processing technologies. Two areas of our work are described: (*a*) garlic cultivated with selenium fertilization and (*b*) foods high in conjugated linoleic acid. Both selenium and conjugated linoleic acid are powerful chemopreventive agents in the animal tumor model. The rationale of delivering these two specific compounds through the food system will be developed. Preliminary studies will be summarized to show the feasibility of this approach in suppressing carcinogen-induced mammary cancer in rats. Finally, the advantages of using foods to provide anticarcinogens to the general population as part of a chemopreventive strategy will also be discussed.

Introduction

The principle of cancer chemoprevention has been elegantly delineated by Wattenberg (1, 2). Essentially, there are two distinct strategies associated with the concept: general population chemoprevention and targeted chemoprevention. This presentation is focused on research that could potentially be applied to implement the former strategy, which, by definition, is aimed at providing cancer protective chemicals to large segments of the population who are not at an increased risk because of known exposure to carcinogens, genetic predisposition, or prior diagnosis of malignancy. Although success of this broad-based strategy is difficult to measure, the public health implication is profound. A great majority of individuals in the general population is in reasonably good health, and morbidity from cancer is only one of many maladies that they will succumb to in the aging process. Consequently, there is no compelling need to adopt an aggressive approach in general population chemoprevention. The agents that will be selected should have no toxicity. Because of the intrinsic requirement for a wide distribution in this situation, an expeditious way of delivering these protective agents is through the food system. As a matter of fact, a driving force for general population chemoprevention can be traced to the mounting epidemiological and experimental data which strongly suggested the beneficial effects of certain dietary constituents against cancer. These constituents include micronutrients (vitamins and minerals) as well as minor chemicals which have no nutritional value.

Several governmental and private health institutions have recommended an increased consumption of fruits, vegetables, and grains and a decreased consumption of fat and animal products to reduce cancer prevalence in the United States. These dietary guidelines, although laudable in their own rights, are not without some pitfalls. First, it is inherently difficult to change the eating habits of large segments of the population, especially in an economically affluent society. Second, the question of how much change in the intake of certain food groups is necessary in order to reach the desired outcome is still debatable. Third, personal characteristics such as preference, lifestyle, attitude, and lack of awareness and resolve are all tangible barriers to achieving dietary changes. An alternative is to intentionally enrich food with known cancer protective agents through agricultural methods or food-processing technologies. In this way, people are presented with more choices so that it will be convenient for them to adopt a healthy and prudent diet. In the last few years, we have been working on the chemopreventive properties of certain substances which are naturally part of, or could be added to, food. Two areas of our research, which is still in the infant stage, are described below.

Garlic Cultivated with Selenium Fertilization

Diets high in selenium have been shown to suppress carcinogenesis in many different tumor models (3). Although most investigations of animals used inorganic selenite as the test reagent, it should be noted that selenium is consumed by humans in an organic form in the diet. Little information, however, is available regarding the forms of selenium in common food. The major reason is due to the very low levels of selenium in plant and animal products. Of the few forms that have been identified, selenium occurs as analogues of a variety of sulfur compounds, particularly the sulfur amino acids (4). For example, about half of the selenium in seleniferous wheat is in the form of selenomethionine. In some species of *Astragalus* that accumulate high concentrations of selenium, methylated derivative such as Se-methylselenocysteine has been isolated. In nuts, a major form of selenium is selenocystathionine. Animal tissues, on the other hand, contain selenocysteine as a constituent of a class of proteins known collectively as selenoproteins. Recently, Ip and Ganther (5) delineated the relationship between the chemical form and the anticarcinogenic activity of a variety of selenium compounds. They concluded that the cancer protective efficacy of a selenium-containing compound depends on the availability of selenium for metabolism as well as the location where the selenium moiety enters the metabolic pathway. Based on these criteria, a hierarchy of anticancer potency was established for some of the selenium compounds mentioned above: Se-methylselenocysteine > selenite > selenomethionine. Thus, the chemical form of selenium is clearly an important factor in determining its biological activity.

One of our goals in selenium chemoprevention research is aimed at finding ways of providing sufficient quantities of selenium safely in a food system. Plants are known to convert inorganic selenium in soil to organoselenium analogues of naturally occurring sulfur compounds. Vegetables with a rich source of sulfur might, therefore, be expected to concentrate selenium if cultivated in a medium so fertilized. This idea was tested with garlic, which is abundant in a variety of sulfur compounds. A major reason for choosing garlic as the experimental crop is because the allyl sulfides present in garlic are known to have anticarcinogenic activity (6). By substituting sulfur with selenium, we had hoped to produce more powerful anticancer agents. The hypothesis is supported by the previous research of Ip and Ganther (7) with structurally related selenium and sulfur analogues in which they showed that, molecule for molecule, selenium is much more active than sulfur in cancer prevention. Garlic crops high in selenium were successfully cultivated at Cornell University in Ithaca, NY (8). This garlic, grown in a selenium-fertilized medium and with a final selenium content of about 150 ppm dry weight, is hereafter designated selenium-enriched garlic. We recently reported that this selenium-enriched garlic is indeed superior to regular garlic or selenite in the

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[2] To whom requests for reprints should be addressed, at Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263.

suppression of mammary tumors in an animal model (8). Furthermore, the selenium from this garlic is capable of maintaining maximal activity of functional selenoenzymes such as glutathione peroxidase and type I 5′-deiodinase at nutritional levels of selenium intake (9). Our study therefore shows that selenium-enriched garlic represents a feasible way of delivering selenium as both an essential nutrient and an anticarcinogen.

It could reasonably be argued that the selenium-enriched garlic most likely contains several selenium compounds that might account for these desirable attributes. If so, this illustrates why the approach of delivering selenium through a food system is an attractive one. Garlic contains an abundance of sulfur amino acids and their derivatives, as well as a variety of alkyl and alkenyl cysteine sulfoxides. The latter compounds are the precursors responsible for the pungent odor of garlic when it is crushed or sliced. Through a cascade of enzymatic and chemical reactions, allyl sulfides and disulfides are produced from the sulfoxides. Not only do these volatile sulfides contribute to the flavor of garlic but they are also active anticancer agents. When garlic is grown in a selenium-fertilized medium, selenium analogues of many of these sulfur-containing compounds are expected to be formed. At the present time, there is no information concerning which of these selenium-substituted compounds are responsible for the nutritional or anticarcinogenic activities in selenium-enriched garlic. A priority of this research is to characterize the chemical composition of selenium in the garlic. It is interesting to note that tissue selenium levels are actually lower in animals ingesting the selenium-enriched garlic than in those fed a similar amount of selenium from sodium selenite (8). This finding suggests that the selenium from garlic is probably excreted at a faster rate, but the lower tissue retention does not diminish the anticancer efficacy of the selenium from garlic. The reduced tissue accumulation of selenium from selenium-enriched garlic may alleviate the concern of selenium toxicity at high levels of supplementation. Future research in this direction could potentially open a new avenue in delivering selenium compounds which can be consumed safely and have a high cancer chemopreventive activity.

Conjugated Linoleic Acid: A Powerful Anticarcinogen from Animal Fat Sources

CLA[3] is a collective term which refers to a mixture of positional and geometric isomers of linoleic acid (*c*9,*c*12-octadecadienoic acid). The two double bonds in CLA are in positions 9 and 11, or 10 and 12, along the carbon chain, thus giving rise to the designation of a conjugated diene. Each of the double bonds can be in the *cis* or *trans* configuration. CLA, a normal isomerization product of linoleic acid metabolism by rumen bacteria, is a naturally occurring substance in food. It was initially isolated and identified by Ha *et al.* (10) as an anticarcinogenic agent from grilled ground beef and then shown to be present in a variety of foods (11, 12). In general, CLA is higher in meat from ruminants than nonruminants. It is also high in dairy products, but natural and processed cheeses vary substantially in their CLA content, ranging from 3 to 9 mg/g fat. Thus, there could be considerable differences in the dietary intake of CLA, depending on the eating habits of individuals.

Since a wide variety of foods high in CLA are available, our intent is to describe the anticarcinogenc activity of chemically prepared CLA and to advance the notion that foods high in CLA, as part of an overall balanced diet, could play a role in cancer chemoprevention. We have used primarily the DMBA-induced mammary tumor model in female Sprague-Dawley rats for our studies. In the initial experiment reported in 1991 (13), three levels of CLA feeding (0.5, 1, and 1.5% by weight) were started 2 weeks before DMBA administration and continued until the end of the experiment (6 months after DMBA). All rats (n = 30 per group) received 10 mg of DMBA (high dose) by gavage at 50 days of age. Our data indicated that the total number of mammary tumors in the 0.5, 1, and 1.5% CLA groups was reduced by 32, 56, and 60%, respectively. Thus, it appears that maximal inhibition can be achieved with 1% CLA. We have, therefore, set the upper limit of the dose response relationship in this experiment.

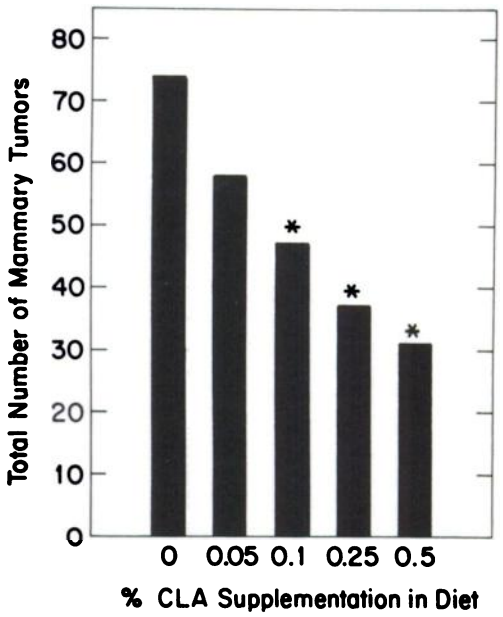

Fig. 1. Mammary cancer prevention by supplementation of different levels of CLA in the diet. Animals were given 5 mg of DMBA i.g. at 50 days of age. There were 50 rats per group. CLA was added to the diet starting 2 weeks before DMBA administration and continued for 9 months until the end of the experiment. *, statistical significance at $P < 0.05$.

In an attempt to expand the CLA efficacy curve below 0.5%, we carried out another experiment similar to the previous one with the exception of two modifications: (*a*) animals were given 5 mg of DMBA (low dose) and (*b*) the sample size per group was increased to 50. The larger sample size ensured adequate statistical power due to the reduced number of tumors produced per rat by the low dose of carcinogen. Rats were fed the CLA-containing diets at 0.05, 0.1, 0.25, and 0.5% starting 2 weeks before DMBA administration and continuing for 9 months. Tumors took a longer time to develop with the low dose of DMBA. As shown in Fig. 1, there was clearly a dose-dependent effect of CLA on mammary cancer inhibition with the dietary levels of 0.05–0.5% ($P < 0.05$ by regression analysis). Total mammary tumor yield was reduced by 22, 36, 50, and 58% in the 0.05, 0.1, 0.25, and 0.5% CLA diets, respectively. Intergroup comparison showed that as little as 0.1% CLA was sufficient to cause a significant reduction in the number of tumors.

In the previous two CLA cancer prevention experiments, CLA was given starting 2 weeks before DMBA administration and then continued for 6–9 months. A third experiment was carried out to see whether short-term CLA feeding from weaning (21 days of age) to the time of carcinogen administration (50 days of age) was able to offer any protective effect against subsequent tumorigenesis. This particular period corresponds to maturation of the rat mammary gland to the adult stage with the number of terminal end buds decreasing gradually and differentiating to alveolar buds and lobules. Two different carcinogens were used for mammary tumor induction: DMBA, which requires metabolic activation, and MNU, which is a direct alkylating agent. Our results in Fig. 2 showed that 1% CLA significantly reduced

[3] The abbreviations used are: CLA, conjugated dienoic derivative of linoleic acid; DMBA, 7,12-dimethylbenz(*a*)anthracene; MNU, methylnitrosourea; i.g., intragastrically.

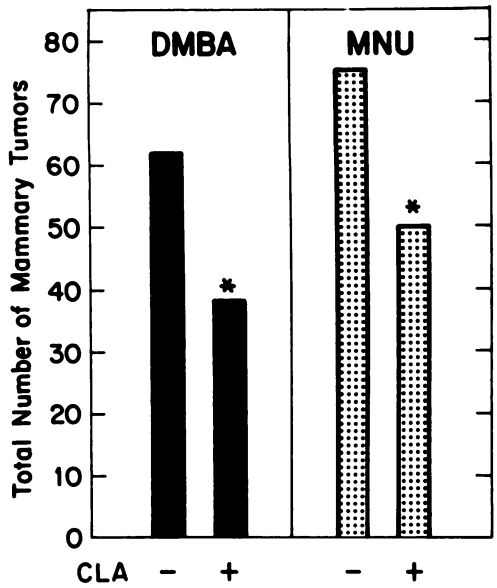

Fig. 2. Mammary cancer prevention by short-term feeding of CLA. Animals were given 10 mg of DMBA i.g. or 6 mg of MNU i.p. at 50 days of age. There were 25 rats per group. CLA was added to the diet at a concentration of 1% starting from weaning until 1 week after carcinogen administration for a total period of 5 weeks. *, statistical significance at $P < 0.05$.

total mammary tumor yield by 39 and 34% in the DMBA and MNU models, respectively. On first glance, it seems that the addition of 1% CLA should have produced a greater inhibitory effect based on the results of the previous two experiments. However, the administration of a high dose of carcinogen for mammary tumor induction in this study (refer to legend of Fig. 2 for doses of DMBA and MNU) coupled with the shorter duration of CLA feeding probably accounted for the attenuated inhibitory response. More importantly, the fact that CLA is suppressive in the MNU model suggests that it may have a direct modulating effect on susceptibility of the target organ to neoplastic transformation. Future research will be focused on testing this hypothesis.

Of the vast number of naturally occurring substances that have been demonstrated to have anticarcinogenic activity in experimental models, nearly all of them are of plant origin (1, 2). CLA is unique because it provides an example that fats from meat and dairy products contain some component that has an attribute in cancer protection. A 300-g rat fed a 0.1% CLA diet will consume about 0.015 g CLA/day. In a direct extrapolation to a 70-kg person, this is equivalent to a daily CLA intake of 3.5 g, a figure slightly higher than the estimated consumption of approximately 1 g/person/day in the United States (11). If the cancer protective efficacy of CLA can be further characterized and its mechanism of action delineated in the near future, there is a good possibility that foods high in CLA, or possibly CLA-enhanced foods, may play a role in cancer chemoprevention. This concept could be particularly appealing to people who desire a food-based approach to cancer prevention without making radical changes in their eating habits.

Discussion

In view of the impossible task of persuading the public to eat only those foods that are presumably good for their health and the need of providing consumers with a variety of food choices, the time has come to enrich our foods with known cancer preventive agents so that their beneficial effects can be realized fully over the life span of an individual. Cancer is a disease which takes many years to develop. A given individual is continuously exposed to a spectrum of mutagenic and promoting agents from the environment, many of which are etiological factors for neoplastic initiation, transformation, and progression. Successful intervention, therefore, depends on the continuous availability of anticarcinogens to prevent, counteract, or even reverse their insults. Foods are a relatively inexpensive and effective way to deliver substances with cancer protective properties. With respect to the pros and cons of food *versus* pill form of delivery, most people do not have the discipline and perseverance to follow a prescription over a sustained period if it is dispensed in pills. The food enrichment method overcomes this hurdle because people will be eating for their health with no extra demand on their effort. Since the principle of general population chemoprevention is not directed at high-risk individuals, the anticarcinogens to be incorporated or enriched in food have no stringent requirement for organ site specificity. Furthermore, both "blocking agents" and "suppressing agents" are equally suitable candidates for this purpose. As defined by Wattenberg (1), blocking agents prevent carcinogens from reaching or reacting with critical target macromolecules (by interfering with activation of carcinogens and/or facilitating their detoxification), whereas suppressing agents retard or even reverse the evolution of the neoplastic process in cells which otherwise would become malignant. In other words, inhibitors with different mechanisms of action can be consumed together by enriching them in discrete and safe quantities in foods. In contrast to packaging chemopreventive agents in a pill form, the approach of delivering them through the food system also minimizes the possibility of overconsumption. The field of cancer chemoprevention has experienced a rapid growth in the identification and characterization of a vast number of anticarcinogenic substances that are present naturally in many of our food sources. A new era challenges our ingenuity to take advantage of this diversity in developing a strategy that utilizes foods high in these compounds as part of an approach toward general population cancer chemoprevention.

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