Natural Agents in the Prevention of Cancer, Part Two: Preclinical Data and Chemoprevention for Common Cancers

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Abstract
This paper is the second of a series examining the use of nutritional supplements as chemopreventive agents. The animal and *in vitro* data are reviewed in support of their use. Human safety data and mechanisms of action are described as well. Many over-the-counter dietary supplements have been shown to have significant chemopreventive activity in preclinical studies. Few side effects are associated with even long-term use of these agents. Along with dietary and lifestyle risk-reducing strategies, nutritional supplementation appears to be a viable intervention for those considered to be at high risk of developing cancer.
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Introduction

This paper is the second of a series examining the use of nutritional supplements as chemopreventive agents. The first paper in the series examined the data from human chemoprevention trials.[1] In the present paper the mechanisms of action of promising treatments will be discussed. *In vitro* and animal data are presented in support of the agents as appropriate. The subject of chemoprevention with nutritional agents has been the subject of voluminous research, and this review should not be considered exhaustive. In cases where review articles already exist regarding a particular agent (e.g., vitamin A, beta-carotene), these papers should be consulted for a more complete summary.

The data presented in this review will focus on three common tumor types: breast, prostate, and colon cancers. While data are available regarding prevention of other tumor types, it is not as extensive as the data covered in this paper. It is the opinion of the authors that agents with a clear record of safety in human studies, evidence of chemoprevention in animal studies, and well-understood mechanisms of action, should be considered for clinical use pending results of large human trials.

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Vitamin A

Vitamin A is obtained from the diet in the form of retinyl esters, which are subsequently de-esterified to retinol. Retinol is then irreversibly oxidized to become retinoic acid. Retinoic acid is the form of vitamin A that binds with nuclear receptor sites and is necessary for the normal growth and differentiation of epithelial tissue.[2] The effects of vitamin A on cellular differentiation are mediated by two separate classes of nuclear receptors, which in turn modify the effects of many compounds, including prostaglandins, vitamin D, and steroid and thyroid hormones.[3] Many studies have examined the effects of isomers of vitamin A, including all-trans retinoic acid, 9-cis retinoic acid, and 13-cis retinoic acid. These isomers are all considered to be interconverted in humans, and may be less hepatotoxic than retinol.

Animal research has demonstrated a chemopreventive effect of retinoids in many types of cancer, including mammary cancer and colon cancer models.[4,5] *In vitro* research has identified a number of promising mechanisms of action, including decreasing serum insulin-like growth factor-1, inhibition of 5-alpha-reductase (the enzyme that catalyzes formation of dihydrotestosterone), and up-regulation of transforming growth factor-beta.[4]

Epidemiological studies on the cancer preventive activity of dietary vitamin A have been inconclusive, perhaps because of confounding factors. Vitamin A is only present in animal foods, and thus dietary vitamin A intake may be a marker for a high meat diet, a risk factor for many cancers. Prospective trials have shown a very modest reduction in breast cancer risk in women with the highest intakes of dietary vitamin A.[6] One prospective epidemiological trial concluded that people taking supplemental vitamin A had a reduced risk of developing breast cancer only if they were in the lowest third of dietary vitamin A intake.[7]

Although the preclinical data have been promising, human studies using vitamin A or retinoids as chemopreventive agents have been largely disappointing.[1] It appears likely from the epidemiological data that the protective effect of retinoids is limited to those who are deficient in dietary vitamin A. It is also possible that the effect is limited to particular clinical situations (e.g., bladder cancer, premenopausal breast cancer).

Carotenoids

Carotenoids are a family of conjugated polyene molecules found largely in fruits and vegetables. Carotenoids are antioxidant, and certain carotenoids can serve as precursors to retinol in humans. Of the more than 600 carotenoids, beta carotene and lycopene have generated the most attention in the chemoprevention field.

As discussed in the first paper in this series,[1] beta carotene has been extensively studied in human trials as a chemopreventive agent. In contrast to the human data, which have largely found beta carotene supplementation to be associated with either no change or an increase in cancer risk, epidemiological evidence has very strongly associated beta carotene intake with reduction in the risk of cancer of many different types.[8] Several schools of thought exist regarding the discrepancy between epidemiology and human experimental data.

The first is that the human studies that used synthetic beta carotene may not have used the right nutrient mixture for chemoprevention.[9] Animal[10] and preliminary human research[11] have shown mixed carotenes have a better chemopreventive action than synthetic beta carotene. Secondly, it has been theorized that beta carotene may have a pro-oxidant effect *in vivo*,[12] an effect that could potentially be carcinogenic. These two theories are not mutually exclusive, and it is possible both are true to some extent.

In vitro and animal studies have demonstrated a number of mechanisms by which beta carotene can inhibit carcinogenesis, including antioxidant activity, vitamin A precursor status, enhancement of gap junction communication, an immunological effect, and induction of hepatic detoxification of carcinogens.[13]

Epidemiological studies have correlated both high intake[14] and high serum concentrations[15] of lycopene with reduced risk of prostate cancer. High adipose concentrations of lycopene have been associated with a reduced risk of breast cancer.[16] Lycopene has been shown to inhibit cancer cell growth *in vitro*, including prostate,[17] breast,[18] and lung[18] cancer cell lines. Animal studies have shown lycopene inhibited development of mammary[19] and colon[20] tumors.

Lycopene's mechanisms of action are somewhat obscure. A human crossover trial found that consumption of tomatoes containing 16.5 mg of lycopene for 21 days led to a 33-percent reduction in the amount of lymphocyte DNA damage sustained after exposure to hydrogen peroxide *ex vivo*.[21] Lycopene has been shown to reduce the growth-stimulating effect of insulin-like growth factor on human breast cancer cells.[22]

Folic Acid

Expression of genes is controlled in part by DNA methylation. Hypomethylation in the presence of folic acid deficiency has been theorized to be one of the mechanisms by which cancer development can be encouraged.[23] Both low folate status and DNA hypomethylation have been directly observed in squamous cell lung cancer tissue compared to uninvolved bronchial mucosa from the same patients.[24]

Epidemiological studies have shown low folate status to correlate with increased risk of cancers of the cervix, lung, esophagus, brain, pancreas, breast, and especially the colon.[25] The benefits of folic acid may be greatest in those with significant deficiencies, such as in patients taking sulfasalazine (a drug that depletes folate) for ulcerative colitis.[26] Data from the Nurses' Health Study indicate folic acid may at least partially offset the increased breast cancer risk associated with consumption of alcohol.[27] Human chemoprevention trials using folic acid have been performed and were discussed in the first paper in this series.[1]

Vitamin B6

Increased likelihood of recurrence of breast cancer after mastectomy has been correlated in preliminary studies with both poor metabolism of a loading dose of L-tryptophan (indicating a functional deficiency of vitamin B6),[28] and low urinary concentration of 4-pyridoxic acid, a major metabolite of vitamin B6.[29] Dietary intake of vitamin B6 was found not to correlate at all with the risk of breast cancer in a prospective study, however.[30]

Elevated levels of prolactin have been implicated in the pathophysiology of both breast[31] and prostate[32] cancers. Although B6 has been reported to suppress production of prolactin,[33] some studies have failed to find this effect.[34,35]

Vitamin B12

Like folic acid, deficiency of vitamin B12 can lead to hypomethylation of DNA.[24] A role for vitamin B12 in the process of carcinogenesis has been theorized since 1954, when abnormal cell types were found in the stomach lining of patients with pernicious anemia.[36] Women in the lowest quartile of serum vitamin B12 have been found to be at increased risk of developing breast cancer in a prospective, epidemiological study.[30] High mean corpuscular volume (MCV), which is often a sign of either vitamin B12 or folate deficiency, has been found to be predictive for a risk of colorectal polyps in men.[37] Vitamin B12 treatment, together with folic acid, has been shown to reverse a precancerous condition of the lung

called squamous metaplasia[1,38] but has not been used in primary prevention trials to date.

Vitamin C

Since vitamin C is a potent water-soluble antioxidant, it has generated interest as a potential cancer preventive compound. Vitamin C is necessary for the recycling of glutathione, another endogenous antioxidant.[39] It has been theorized to protect against the ability of cancer cells to invade tissue, in part by strengthening the cellular matrix.[40] Vitamin C is directly cytotoxic to certain cancer cell lines *in vitro*. This toxicity can be reversed by the addition of the enzyme catalase into the medium, suggesting that the cell-killing effect of vitamin C is due to a production of hydrogen peroxide within the cell.[41] For most cancers, however, the only information available regarding the chemopreventive activity of vitamin C is epidemiological research.

Retrospective dietary data show that each 300 mg of dietary vitamin C intake is associated with a roughly 30-percent decrease in breast cancer risk.[42] But when the same research group analyzed prospective dietary studies (those that did dietary analysis before the breast cancer diagnosis), no association between dietary vitamin C and breast cancer risk was noted. Another prospective trial found no relationship between plasma levels of ascorbate and risk of breast cancer in the subsequent five years.[43]

Similar to breast cancer, no consistent relationship has emerged from epidemiological studies between dietary vitamin C intake and prostate cancer risk.[44] In one study, subjects taking vitamin C supplements of any dose were found to have a 23-percent decreased risk of developing prostate cancer ($p > 0.05$).[45]

Three of four intervention trials have found a significant benefit from vitamin C supplementation, often along with other interventions, in the treatment of colon polyp patients. In the first of these trials, administration of 3 g/day of vitamin C for nine months led to a significant reduction in polyp area in patients with polyposis coli compared to placebo.[46] In a later trial, supplementation with 30,000 IU vitamin A palmitate, 70 mg d,l-alpha-tocopherol, and 1 g vitamin C per day for six months led to a statistically significant reduction in abnormal cell proliferation in patients with colorectal adenomas.[47] Also, supplementation with 4 g/day vitamin C for four years, in addition to vitamin E (400 IU) and a fiber supplement (22 g/day) was associated with a reduction in polyps in patients with familial polyposis.[48] In the negative trial, supplementation with 1 g/day vitamin C with 400 mg vitamin E for four years was not associated with reduced risk of recurrent colonic adenoma.[49] While the data are not unanimous, evidence exists to support the possibility that oral vitamin C can help reduce the area, proliferation, and recurrence of precancerous colon lesions.

Vitamin D

Vitamin D is a molecule with hormonal activity that is best known for its effects on calcium metabolism. The metabolism and safety of vitamin D has been recently reviewed,[50] with the conclusion that supplementation with vitamin D is likely to be safe up to levels approaching 10,000 IU/day. In addition to its role in calcium metabolism, vitamin D appears to be important in regulation of cellular growth and differentiation. Vitamin D has been shown to cause G1 cell cycle arrest in prostate cancer cells, mediated through direct effects on p21, p27, and E-cadherin.[51] Cells expressing vitamin D receptors have been found in human tumor lines, including breast,[52] prostate,[53] and colon.[54] Vitamin D has been shown to reduce tumor secretion of type IV collagenases,[55] and thus to reduce the number of metastases in an animal study.[56]

Epidemiology has long suggested a role for suboptimal vitamin D levels in the risk of common tumor types. Exposure to low levels of UV light from the sun is thought to

account for about six percent of the U. S. variation in prostate cancer mortality.[57] Low prediagnostic serum levels of vitamin D have been correlated with increased risk of poorly differentiated prostate tumors.[58] Women with breast tumors that express vitamin D receptors (over 80 percent of tumors) were found to have a significantly longer disease-free survival than women whose cancers did not express this receptor.[52]

Vitamin D has been shown to reduce the proliferation of many tumor cell lines *in vitro*, including breast,[52] prostate,[53] and colon.[54] Treatment of animals with synthetic vitamin D analogues has been shown to inhibit metastasis and prolong survival time in breast cancer models.[59] Vitamin D has been shown to inhibit the development of colon tumors in animals, an effect the authors attributed partially to angiogenesis inhibition.[60] Treatment of men having recurrent prostate cancer with between 0.5 and 2.5 mcg of oral calcitriol at bedtime (hypercalcemia was the dose-limiting side effect) caused a significant slowing of the rate of prostate specific antigen (PSA) increase compared to pretreatment levels in six of seven patients.[61] In the seventh patient, a non-significant trend toward reduction in the rate of PSA elevation was noted. In another preliminary study, 44 percent of patients with hormone-refractory advanced prostate cancer and bone metastases were found to have low serum concentrations of vitamin D.[62] In this trial, supplementation with 2000 IU vitamin D2 was associated with reduced bone pain and improved quality of life from baseline.

Vitamin E

Several human intervention trials have examined the ability of vitamin E to prevent carcinogenesis. Vitamin E, often as part of a larger nutritional protocol, has been found to significantly reduce incidence of prostate, bladder, and stomach cancers, as well as to prevent recurrence of colonic adenomas in some, but not all studies.[1] Both the Women's Health Study[63] and the Physician's Health Study II[64] are ongoing clinical trials with large patient populations following up on the promising results of the preliminary vitamin E chemoprevention trials.

The subject of vitamin E in cancer prevention has been reviewed previously.[65,66] These reviews discuss the extensive animal and *in vitro* research in support of vitamin E in cancer prevention. Several mechanisms of vitamin E are considered important in this regard, including stimulation of wild-type p53 tumor suppressor gene, down-regulation of mutant p53, activation of heat shock proteins, and an antiangiogenic effect mediated by blockage of transforming growth factor-alpha (TGF-α).[65]

Selenium

Administration of 200 mcg selenium from yeast has been shown to reduce the incidence of several types of cancers in a human trial.[1,67] These data from the intervention trial confirm prior epidemiological studies that have often shown low selenium status to be associated with increased total cancer incidence.[68,69] In these studies, the inverse association between selenium and gastrointestinal, prostate, and lung cancers appears to be particularly strong. The relationship between selenium status and breast cancer risk is less well-defined, with a large geographical study showing clear inverse correlation in risk,[70] but a prospective trial showing no significant relationship between toenail selenium levels and breast cancer risk.[71] Animal studies using a variety of different tumorigenesis models have largely found selenium to have significant chemopreventive activity.[72]

Although there are several proposed mechanisms of action for selenium, including induction of glutathione peroxidase, modulation of cytochrome p450 systems, and immune modulation,[73] the most important mechanism(s) probably remain elusive. Similarly, the most effective form of selenium

Table 1: Nutrients in the Chemoprevention of Cancer

Nutrient	Proposed Mechanisms
Vitamin A	Inhibition of insulin-like growth factor-1; inhibition of 5-alpha-reductase; upregulation of transforming growth factor-beta.
Carotenoids: Beta Carotene	Antioxidant; vitamin A precursor; enhanced gap junction communication; induction of hepatic detoxification of carcinogens
Carotenoids: Lycopene	Protect DNA from H_2O_2-induced damage; decrease growth-stimulating effect of insulin-like growth factor
Folic Acid	DNA methylation for normal gene expression
Vitamin B12	DNA methylation for normal gene expression
Vitamin C	Antioxidant necessary for the recycling of glutathione; strengthening the cellular matrix preventing metastases; directly cytotoxic to certain cell lines in vitro, possibly due to production of hydrogen peroxide within the cell.
Vitamin D	Important in cellular growth and differentiation; G1 cell cycle arrest; reduce tumor secretion of type IV collagenases (reducing metastases)
Vitamin E	Stimulation of wild-type p53 tumor suppressor gene; antiangiogenesis; down regulation of mutant p53

supplementation has not been definitively demonstrated. While the majority of the animal chemoprevention studies have used inorganic selenium, the most successful human trial used organic selenium from yeast. Yeast-based selenium is approximately 40-percent selenomethionine, 20-percent other amino acid conjugates (e.g., selenocysteine, methylselenocysteine), and 40-percent unidentified selenopeptides.[74] In one animal study, co-administration of vitamin C nullified the chemopreventive effect of inorganic selenium (selenite), but not that of selenomethionine.[75]

Calcium

A human intervention trial found that supplementation with 1200 mg calcium carbonate per day led to a significant reduction in colonic adenoma recurrence risk.[76] Both men and women with high dietary intakes of calcium have been found to have a lower risk of developing colon cancer.[77] Calcium has been found to protect against colon carcinogenesis in animal models as well.[78] Although the mechanism by which calcium appears to protect against colon cancer formation is not entirely clear, it appears calcium may precipitate toxic bile acids in the colonic lumen, thus reducing the rate of proliferation of colonic epithelium.[79]

High calcium concentrations in drinking water have been correlated with a significantly reduced risk of developing breast cancer,[80] but not prostate cancer[81] in Taiwan. Animal studies have not yet clarified a role for calcium in breast and prostate cancer prevention.

Zinc

The zinc concentration of cancerous prostate tissue (146 mcg/g) has been found to be significantly lower than that of normal prostate tissue (1018 mcg/g) and BPH prostate tissue (1142 mcg/g).[82] Whether this finding is a cause or an effect of neoplastic transformation is still debated. Zinc has been found to inhibit the growth of prostate cancer cell lines *in vitro*.[83] Cell cycle arrest (G2/M) was noted in lines that had mutated or wild-type (normal) p53 tumor suppressor gene. Men who take supplemental zinc were found to have a borderline-significant 45-percent reduction in prostate cancer risk in a case-control study.[84] Zinc inhibits the activity of prostatic 5-alpha-reductase and may inhibit prostatic uptake of the potential carcinogen cadmium.[85]

Diets containing high concentrations of zinc, with or without high levels of copper, were found to be ineffective in preventing mammary cancers in an animal study.[86] Serum zinc concentrations have been found to be mildly reduced in patients with breast cancer and slightly elevated in patients with colon cancer compared with healthy controls.[87] Another study found a strong positive correlation between high serum zinc concentrations and breast cancer.[88]

Table 1 summarizes the cancer preventive potential of vitamins and minerals.

Coenzyme Q10

Coenzyme Q10 (CoQ10) is a lipid-soluble antioxidant involved in the production of ATP via the electron transport chain. Tumor tissue levels of this nutrient have been found to be significantly lower in breast cancers than in surrounding normal tissue.[89] A series of preliminary reports suggest a potent treatment effect of CoQ10 in advanced breast cancer.[90,91] These reports have methodological flaws that make them difficult to interpret, however. An unpublished human trial found CoQ10 treatment to cause regression of prostate tumors, as well.[92] Colon cancer tissue contains significantly higher levels of CoQ10 than surrounding tissues, for reasons that are unclear.[93] Future animal and human studies will need to elucidate the role of CoQ10 in prevention and treatment of cancers.

Quercetin

Quercetin is a flavonoid present in many foods of plant origin. The anticancer mechanisms of quercetin have been reviewed in a recent issue of this journal.[94] Quercetin has been shown to have a preventive effect in a number of different animal tumor models, including oral cancer,[95] fibrosarcoma,[96] skin,[97] mammary,[98] and multi-organ tumorigenesis.[99] Colon cancer models have yielded conflicting results, with studies that show decreased risk,[100] no change,[101] or even increased risk.[102] Quercetin was found to be superior to

tamoxifen *in vitro* as a growth inhibitor of the estrogen-receptor negative MCF-7 breast cancer cell line.[103]

Although early research on quercetin showed a minimal absorption of the compound, newer research has refuted these findings.[94] Quercetin chalcone has been proposed as a more absorbable form of quercetin due to its increased water solubility, but has not undergone human trials to demonstrate this ability. Quercetin chalcone administration has been shown to slow the growth of human colon tumors transplanted into mice.[104]

cell culture study found a synergism in effect on cellular differentiation between curcumin and either the active form of vitamin D or all-trans retinoic acid, although curcumin alone had no significant effect.[109] Curcumin also shows synergy with genistein in its ability to reduce the proliferative effect of estrogenic pesticides on estrogen-receptor positive breast cancer cells.[110]

Rats eating a diet containing 2000 ppm curcumin developed colon tumors at a significantly reduced rate compared to controls.[111] Curcumin's tumor inhibiting effect is similar

Figure 1: Curcumin
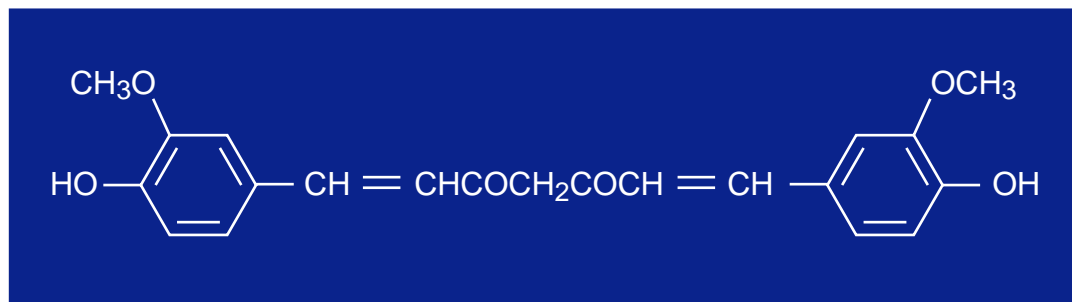
Curcumin

Curcumin (Figure 1), a compound found in turmeric (*Curcuma longa*), has a number of potential cancer-preventing mechanisms of action. The first is its inhibitory effect on the proinflammatory enzymes cyclooxygenase and lipoxygenase.[105] A study found the anti-inflammatory efficacy of curcumin to be superior to indomethacin. Curcumin has also been found to induce G2/M phase cell cycle arrest in human colon cancer cells independently of its control of prostaglandin synthesis.[106] Preliminary animal evidence suggests curcumin may have effects on the ability of carcinogens to form DNA adducts[107] and on cytochrome p450 metabolism,[108] but the importance of these mechanisms is not clear. One

to many of the NSAIDs, including aspirin, ibuprofen, and indomethacin.[112] A diet containing two-percent curcumin by weight reduced the percentage of animals developing colon cancers from 40 percent to zero.[109] Administration of curcumin blocked the induction of mammary carcinogenesis by radiation.[113] Curcumin has also been found to inhibit chemically-induced mammary cancers in some,[114] but not all[115] studies. The effect of curcumin on prostate carcinogenesis has not been evaluated in animal studies.

Green Tea

The catechins contained in green tea, which make up about 30 percent of the dry weight of tea leaves, have several anticancer activities. The catechins are oxidized in the

manufacture of black tea, and thus black tea would not be expected to have similar actions. One of the main catechins in tea is epigallocatechin gallate (EGCG), and many studies have focused specifically on its actions. EGCG has been shown to induce G2/M cell cycle arrest and to block the tumor-promoting activities of epidermal growth factor and NF-kappaB *in vitro*.[116] In a human study, 10 of 14 subjects drinking up to 1.8 grams of dissolved green tea solids had a greater than 50-percent reduction in rectal concentrations of PGE2, a prostaglandin associated with tumor promotion.[117] The *in vitro* antimutagenic activity of green tea catechins has been demonstrated to be greater than that of many antioxidants, including curcumin, vitamins C and E, quercetin, glutathione, and N-acetylcysteine.[118] Green tea has been shown to inhibit release of TNF-α, a suspected tumor promotor and a cytokine suspected to be involved in the pathogenesis of cachexia.[119,120] Green tea polyphenols have been shown to inhibit glucuronidation of estrogens *in vitro*, but to mildly stimulate this metabolic pathway *in vivo*.[121] Glucuronidation is a major hepatic conjugation pathway by which estrogens are removed from circulation. Green tea catechins have been shown to inhibit the actions of 5-alpha-reductase and ornithine decarboxylase, two enzymes considered important in the development of prostate cancer.[122] Human studies suggest that serum concentrations of green tea compounds mimicking those used in animal cancer prevention studies can be achieved by drinking approximately five cups of green tea per day.[123]

Green tea compounds have been found to inhibit chemically-induced carcinogenesis of several sites, including mammary[124] and colon[125] cancer models. Injection with EGCG was shown to significantly inhibit the growth of human prostate tumors transplanted into athymic mice.[126] After resection of stage I or stage II breast tumors, Japanese women drinking five cups or more of green tea per day were 44-percent less likely to have tumor recurrence than women drinking four cups or less.[127] No influence on prognosis was noted in women with stage III breast cancer, however. A study of 8,000 Japanese adults found those with the highest consumption of green tea had a reduced total cancer risk, particularly in women.[128]

N-Acetylcysteine

N-acetylcysteine (NAC), a thiol-containing amino acid derivative, is a precursor to glutathione synthesis. Due to its relative lack of toxicity and impressive results in preclinical studies, NAC has generated interest as a chemopreventive agent. Human trials have shown that NAC administration is associated with reduced proliferative index of colonic mucosa in patients with a history of colon polyps,[129] but not with prevention of lung cancer recurrence.[130] NAC has several theorized mechanisms by which it could prevent cancer occurrence and spread, including replenishing of glutathione stores,[131] prevention of DNA damage due to environmental exposures,[132] and inhibition of type IV collagenase stimulation of invasion and metastasis.[133]

NAC has been shown to prevent colon carcinogenesis in animal studies,[134] but did not prevent mammary cancer occurrence in a rat model.[135] NAC has not been studied as a preventive agent for prostate cancers. It is suggested NAC be used with caution in patients undergoing chemotherapy, as some evidence suggests it can reduce the effectiveness of certain cytotoxic drugs, including alkylating agents.[136]

Indole-3-Carbinol

Indole-3-carbinol (I-3-C) is a component of foods of the Brassica family (i.e., broccoli, kale, brussels sprouts, cauliflower). Human studies have shown that addition of either 500 grams per day of broccoli or 400 mg per day I-3-C to the human diet is associated with a significant increase in the urinary ratio

of 2-hydroxyestrogens (2-OH):16-hydroxyestrogens (16-OH).[137] Low urinary 2-OH:16-OH ratios have been associated with increased risk of breast cancer.[138] I-3-C has been shown to arrest the cell cycle in the G1 phase and to increase expression of the p21 and p27 genes.[139] A major metabolite of I-3-C has been shown to be an antagonist of estrogen binding *in vitro*.[140]

A human dose-ranging study concluded that 300 mg I-3-C per day is the minimum dose necessary for chemoprevention.[141] In this and other human studies adverse events were rare and mild. In addition to breast cancer prevention, I-3-C has generated interest in the prevention of cervical cancer.[142] A preliminary study showed indole-3-carbinol reversed some cases of cervical dysplasia.[143] Animal studies have shown a chemopreventive effect in both cervical[144] and breast cancer[145] models. An *in vitro* study showed I-3-C and tamoxifen work by different signaling pathways, and thus represent a potential combination treatment in estrogen receptor-positive breast cancers.[146]

Animal studies have shown significant chemoprevention in chemically induced tumors of the colon,[147] lung,[148] and stomach.[149] I-3-C has been shown to both inhibit[150] and enhance[151] hepatic carcinogenesis, depending on the model used. A phase I human trial showed I-3-C administration arrested or slowed growth of respiratory papillomas (a pre-cancerous condition) in 12 of 18 subjects.[152]

I-3-C is an unstable molecule, and undergoes oligomerization in the gut to a number of different compounds, including diindolylmethane. Diindolylmethane itself has been shown to be chemopreventive in animal breast cancer models.[153] Dose-ranging studies have not been performed in humans with diindolylmethane, however. It is also not clear whether all of the effects seen with I-3-C are mediated by conversion to diindolylmethane.

Inositol Hexaphosphate

Inositol hexaphosphate (IP6) (Figure 2), also known as phytate or phytic acid, is ubiquitous in plant foods. It is present in particularly high concentrations in cereals and legumes. IP6 was first identified as an "anti-nutrient"— a compound able to block absorption of many minerals. Once inside the cell, IP6 is dephosphorylated to a number of lower inositol phosphates (e.g., IP1-5) that play important roles in signal transduction.[154] Cell culture studies found IP6 to have several beneficial genetic effects, including down-regulation of mutant p53 and up-regulation of wild-

Figure 2: Inositol Hexaphosphate
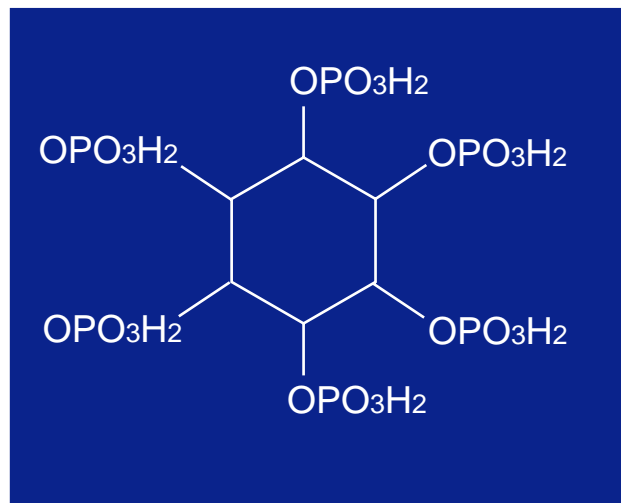
type p53 and p21.[155] These two genes are both thought to be very important in the expression of the malignant phenotype. *Ex vivo* studies with human neutrophils have shown IP6 enhanced the immune response to specific stimuli without direct activation of non-specific inflammatory pathways.[156] None of these mechanisms have been confirmed by clinical trials.

In animal models IP6 prevented chemically-induced colon carcinogenesis.[154] In these studies, doses as low as 0.1-percent IP6 in drinking water had significant effect.[157] Addition of 15 mM IP6 and 15 mM inositol to the drinking water of rats led to significant inhibition of chemically-induced mammary carcinogenesis.[158] The tumors that formed in the treated rats were also smaller than those in the control group. IP6 has been theorized to be at least in part responsible for the observed relationship between a high-fiber diet and reduced risk of breast cancer in humans.[159]

Animal and *in vitro* studies have also shown antitumor effects of IP6 in leukemia, hepatocellular carcinoma, lung cancer, prostate cancer, papilloma, and fibrosarcoma.[160] Human data regarding the safety and efficacy of IP6 are scarce, and are largely limited to epidemiology. The mineral-binding activity of IP6 suggests it should be given either away from meals or in a program including a multivitamin/mineral supplement.

Soy

Soybeans, and particularly the isoflavones contained in soy, have generated interest as chemopreventive agents. Although no clinical trials have been completed to date, several preclinical studies have examined the effects of soy in different tumor types. Multiple mechanisms of action have been demonstrated, including protease inhibition,[161] antimitotic effect,[162] antiangiogenesis,[163] inhibition of protein tyrosine kinase activity,[164] and 5-alpha-reductase inhibition.[165] The most celebrated mechanism of action of soy, however, is its binding at the estrogen receptor site. Soy exhibits a biphasic effect on estrogen receptors, acting as an agonist at low concentrations and an antagonist at higher levels.[166]

Soy has been found to alter estrogen metabolism in both pre- and postmenopausal women. Consumption of 65 mg isoflavones per day by postmenopausal women was found to increase serum 2-OH:16-OH estrone ratios.[167] Urinary excretion of 2-OH estrone was increased by consumption of 158 mg/day soy isoflavones for an entire menstrual cycle in premenopausal women.[168] Consumption of 154 mg/day soy isoflavones for one menstrual cycle was associated with a 25-percent reduction in circulating 17-beta-estradiol concentrations in premenopausal women.[169]

Some research has shown, however, that soy isoflavones have an estrogenic effect on the normal breast. Clinical trials found consumption of 45 mg isoflavones increased the proliferation of normal breast tissue.[170,171] Supplementation with 38 mg per day of the isoflavone genistein was found to increase the secretion of breast fluid in healthy pre- and postmenopausal women, a finding suggestive of an estrogenic effect.[172]

Preliminary *in vitro* studies suggest soy may attenuate the estrogen-receptor antagonist activity of tamoxifen.[173] Soy has been found to be ineffective in preventing hot flashes in breast cancer survivors.[174]

Men who drank soy milk more than once per day had a 70-percent reduction in prostate cancer risk compared to non-soy milk drinkers.[175] Soy isoflavones have been found to inhibit the growth of prostate cancer cells *in vitro*,[176] and in some,[177,178] but not all animal studies.[179] Studies have not been performed to demonstrate the effect of soy on the normal human prostate. Although *in vitro* studies show soy isoflavones inhibited colonic cancer cell growth, very little experimental or epidemiological data point to a significant preventive effect of soy on colon cancer.[180]

Glucaric Acid

D-glucaric acid is a chemical found in many fruits and vegetables in amounts ranging from 100 mg to 3 grams per kilogram.[181] After ingestion, some D-glucaric acid is converted to D-glucaro-1,4-lactone (1,4-GL), which appears to be the active compound (Figure 3). D-glucaro-1,4-lactone inhibits beta-glucuronidase in the colonic microflora.[181] This

Figure 3: Gut Metabolism of Glucaric Acid

Calcium D-Glucarate

↓ (Stomach)

Glucaric acid

↓

D-glucaro-6,3-lactone

↕

*D-glucaro-1,4-lactone

inhibits beta-glucuronidase
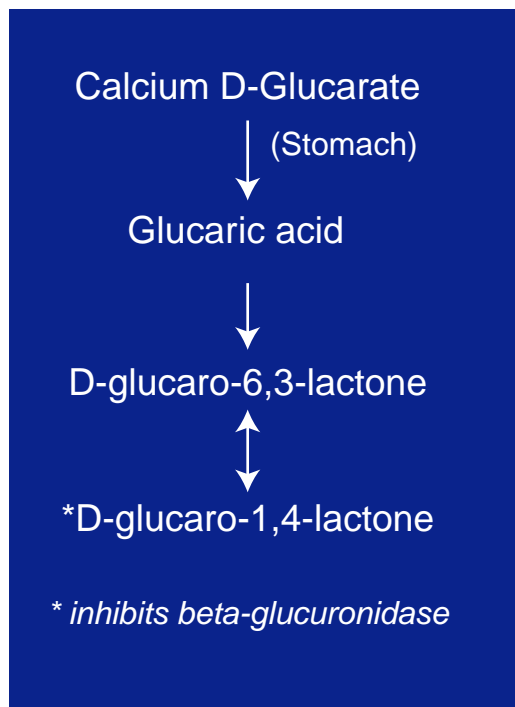
action theoretically reduces the amount of conjugated estrogens and other steroid hormones that are deconjugated in the gut and returned to circulation. It has been theorized that 1,4-GL also has systemic activity, possibly regulating cholesterol and steroid synthesis.[182]

Oral administration of calcium D-glucarate (a calcium salt of D-glucaric acid) led to an inhibition of chemically induced mammary carcinogenesis in rats.[183] Serum estrogen levels were 23-percent lower in rats receiving the calcium D-glucarate. The 4.5 mM/kg doses of calcium D-glucarate used in this trial were very large, however. To approximate this dose in a 70 kg human, 78 grams would be required.

Other animal trials found D-glucaric acid had chemopreventive activity in colon, lung, liver, and skin cancer models.[184] The doses used in these studies were either 70 or 140 mM/kg of diet. Assuming a 2 kg per day diet in a human, this would imply an active dose of roughly 35 to 70 grams. While the

mechanism of action and preclinical data regarding glucaric acid derivatives are promising, until human safety and efficacy data are available these compounds should be used with caution.

Serenoa Repens (Saw Palmetto)

Saw palmetto is an herb that has been used to treat symptoms of benign prostatic hyperplasia (BPH). Its efficacy has been shown to be similar to that of finasteride, a 5-alpha-reductase inhibitor.[185] Although saw palmetto extracts have 5-alpha-reductase inhibitory activity *in vitro*, studies conflict regarding the *in vivo* effect of the herb on dihydrotestosterone levels.[186,187] Saw palmetto has also been shown to reduce prostatic concentrations of epidermal growth factor[181] and to inhibit the growth stimulating effect of prolactin in the prostate.[188]

Although the mechanisms of action of saw palmetto could plausibly reduce the risk of prostate cancer, this effect has not been studied in clinical trials. Saw palmetto is a key component of the herbal formulation PC-SPES, a Chinese herbal formula that has been shown in preliminary human research to have activity against androgen-independent prostate cancer.[189]

Rosemary

Dietary treatment with one-percent rosemary methanol extract inhibited mammary tumor formation in rats.[190] Treatment of animals with rosemary increased 2-OH estrone, reduced 16-OH estrone, and stimulated glucuronidation of estrogen in the liver.[191] It is not currently known, however, whether these effects are possible to duplicate in humans using tolerable doses of rosemary extract.

Conclusion

Many promising preclinical studies have been performed on the efficacy of nutritional interventions for cancer prevention. Some of these studies are currently being

followed up with clinical trials. Others hopefully will follow.

It is the opinion of the authors that agents with a good record of safety may be considered for use before results of large-scale clinical trials are available. An agent with evidence of safe use in humans can be considered for therapeutic use if it has epidemiological, animal, and *in vitro* data in support of efficacy. Many of the agents discussed above meet these criteria.

As discussed in the first paper in this series, preliminary human data show nutritional interventions can have cancer preventive efficacy similar to pharmaceutical options in some cases. Toxicity from these agents tends to be much lower than drug therapies, and health benefits in other areas (e.g., decreased cardiovascular disease risks) may occur.

Nutritional supplementation is no substitute for dietary and lifestyle risk reductions. Physicians interested in cancer prevention should look into the risk factors for the common tumor types and counsel their patients accordingly. For the patient known to be at high risk for cancer, nutritional protocols based on the data presented should be considered.

Acknowledgements

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