Prevention and Treatment of Cancer with Indole-3-Carbinol

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Abstract
Indole-3-carbinol (I-3-C) is a naturally occurring constituent of many plant foods. Oral administration of I-3-C has been shown to manipulate estrogen metabolism in humans in a possibly beneficial manner. I-3-C increases the 2/16-hydroxyestrone ratio, a ratio found to be predictive of breast cancer risk in some prospective studies. Animal and *in vitro* studies have identified a number of other possibly beneficial effects of I-3-C and its metabolites, including inhibition of estrogen binding and modulation of oncogene expression. A chemopreventive effect of I-3-C has been demonstrated in a number of animal models. Some chemical carcinogenesis models have found a tumor promoting effect of I-3-C, however. Epidemiological studies support the hypothesis that high intakes of I-3-C may have broad chemopreventive effect. Preliminary human trials have demonstrated that I-3-C is well tolerated and has a sustained estrogen modifying effect. I-3-C is a good candidate for clinical trial in women at increased risk of developing breast cancer.
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Introduction

Indole-3-carbinol (I-3-C) is a compound found in high concentrations in Brassica vegetables, including broccoli, cauliflower, Brussels sprouts, and cabbage. It has received attention in recent years as a promising preventive and treatment agent for breast and other types of cancers.

I-3-C (Figure 1) has recently become available as a nutritional supplement. Preliminary studies have examined its safety and attempted to elucidate an optimal dose. Early studies have been promising, and will likely be followed up with larger clinical trials. This paper will examine the evidence supporting the use of I-3-C for prevention and treatment of cancer from human, animal, and *in vitro* studies. It will also review the metabolism and safety of oral I-3-C.

Absorption/Metabolism

Animal studies have shown that intravenous or intraperitoneal administration of I-3-C does not have the effect that is seen with oral dosing.[1] This suggests that other metabolic products are largely responsible for the action of I-3-C. Two metabolic products of I-3-C, diindolylmethane (DIM) and indolylcarbazole (ICZ) have received the most attention as active

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Figure 1. Indole-3-Carbinol

constituents, although there are a number of other metabolites of I-3-C that have yet to be fully investigated.[2] Another major metabolic product, 2-(indol-3-ylmethyl)-3,3'-diindolylmethane (LTr-1, also referred to as BII), has recently become of interest.

Following oral administration of 400 mg I-3-C to humans, serum levels of DIM reached between 0.1 and 0.4 mcg/mL.[3] Serum I-3-C was not measurable at this dosage. Presence or absence of other products, such as LTr-1 or ICZ was not reported.

Animal studies have shown DIM and LTr-1 to be the major serum metabolites following oral administration of I-3-C,[4] although other metabolites are measurable in smaller amounts on high performance liquid chromatography (HPLC). DIM and LTr-1 levels were roughly equivalent, with different metabolites being more prevalent in different organs. Another study quantified hepatic concentrations of I-3-C metabolites, finding 24-perent DIM, 20-percent LTr-1, 24-percent of another metabolite called 1-(3-hydroxymethyl)-indolyl-3-indolylmethane, and a number of minor metabolites.[5] In this latter study, the potent metabolite ICZ was found in very small concentrations, at an order of magnitude smaller than the major metabolites.

Different conditions in the gastrointestinal tract may favor production of different metabolites. Formation of LTr-1 is pH dependent, with declining production at pH above 4.5.[4] DIM production increases at pH levels above 3.[4]

Initially, the major route of elimination of I-3-C metabolites is urinary. After 40 hours of continuous dietary administration, however, the fecal route becomes prevalent.[5] I-3-C metabolites have a serum half-life greater than 48 hours after a week of continuous administration in animal studies.[5]

In vitro/In vivo Mechanisms of Action

Indole-3-carbinol has a number of potential mechanisms of action for chemoprevention of cancer. It was one of only eight compounds (including ascorbic acid, vitamin E succinate, and folic acid) found to have benefit in six different *in vitro* chemoprevention models in a National Cancer Institute screening study.[6] While many mechanisms have been described, it is possible others exist, particularly among the minor I-3-C metabolites that have not been well studied.

Several studies have examined the effects of I-3-C and its metabolites on breast cancer cell lines. I-3-C and tamoxifen have been shown to act separately and/or cooperatively to inhibit the growth of estrogen receptor-positive (ER+) breast cancer cells.[7]

Figure 2. Diindolylmethane (DIM)

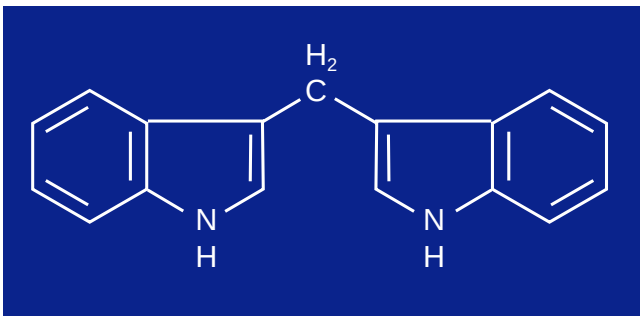

Figure 3. Indolylcarbazol (ICZ)

Tamoxifen and I-3-C appeared to function by different mechanisms. I-3-C can stimulate apoptosis in estrogen receptor-negative human breast cancer cell lines as well.[8]

DIM (Figure 2) has been shown to inhibit proliferation of human breast cancer cells at concentrations achievable through oral supplementation with I-3-C (10-50 microM).[9] Incubation of human breast cancer cells with DIM has been found to stimulate apoptosis.[10] In this study, the apoptosis-promoting activity of DIM was found to be p53 independent.

Figure 4. 2-(indol-3-ylmethyl)-3,3'-diindolylmethane (LTr-1, also referred to as BII)

Many of the I-3-C metabolites have anti-estrogenic activity. Both I-3-C[11] and ICZ[12] (Figure 3) compete with estrogen for binding sites. DIM has been shown to selectively bind to the estrogen receptor, and may act as an estrogen antagonist at physiological concentrations.[13]

LTr-1 (Figure 4) has been shown to inhibit the growth of estrogen receptor-positive and -negative breast cancer cells *in vitro*.[14] This metabolite was found to have estrogen-antagonizing activity that may be at least partially responsible for the effect in ER+ lines.

Other research has focused on the ability of I-3-C metabolites, specifically DIM and ICZ, to induce the cytochrome p450 isoenzymes, CYP1A1 and CYP1A2.[15,16] This induction appears to be mediated by binding of these compounds at the aryl hydrocarbon receptor. Another metabolite, LTr-1 was also found to increase expression of cytochrome p450 isoenzyme CYP1A1.[14] This induction of CYP1A1 appears to be responsible for the estrogen-modulating activity of I-3-C products. No hormonal modulatory effect of oral I-3-C was seen in women with a rare mutant form of CYP1A1 (a mutation that increases breast cancer risk ten-fold), inferring that CYP1A1 manipulation may be an important mechanism of action.[17]

The effect of I-3-C metabolites on the cytochrome p450 isoenzymes is not entirely clear, however. One *in vitro* study showed both I-3-C and DIM lacked significant induction of CYP1A1.[18] I-3-C may also down-regulate certain isoenzymes, including CYP1B1.[2]

I-3-C has been shown to inhibit the ability of human breast cancer cells to invade surrounding tissue.[19] This effect was thought to be mediated through up-regulation of tumor suppressor gene PTEN and adhesion molecule E-cadherin. It is not currently known if DIM or LTr-1 have this effect.

I-3-C has been shown *in vitro* to block the estrogenic stimulation of human papilloma virus (HPV) expression.[20] HPV is implicated in the pathogenesis of cervical and head and neck cancers.

I-3-C induced apoptosis and led to cell cycle arrest at the G1 checkpoint in human prostate cancer cell lines at concentrations between 25 and 100 microM.[21] I-3-C appeared to induce expression of the p21 and p27 tumor suppressor genes, as well as down-regulating NF-kappa-B.

I-3-C has been shown to reduce the activity of the tumor-promoting enzymes ornithine decarboxylase (ODC) and tyrosine kinase at *in vitro* concentrations between 250 and 1000 microM.[22] Perhaps secondary to its effect on ODC, I-3-C has been shown to inhibit cell cycle progression at G1.[22] I-3-C has been shown to increase p21 and p27 expression in MCF-7 breast cancer cells.[23] It is currently unclear whether any of these effects on enzymes and genes are important at concentrations of I-3-C achievable in the serum of humans, although ODC inhibition has been observed in animal studies.[24]

Dietary I-3-C may have immunomodulatory activity as well. Administration of I-3-C at either 50 mg/kg or 150 mg/kg increased delayed-type hypersensitivity reactions in the rat.[25] The higher dose also significantly impaired natural killer (NK) cell function, while the lower dose increased NK activity insignificantly. It is not currently known whether I-3-C has immunomodulatory activity at levels more closely resembling human doses.

I-3-C, and to a lesser extent DIM, have been shown to induce aryl hydrocarbon hydroxylase in animal studies.[26] This enzyme can inhibit the carcinogenic activity of chemicals used to induce cancers in animals, but its role in protecting against human tumors is uncertain.

2:16-Hydroxyestrone Ratio and Cancer Risk

Although the conventional wisdom has proposed that the absolute concentrations of serum estrogens constitute one of the major risk factors in breast cancer genesis, other data have challenged this notion.[27] "The unconventional estrogen hypothesis," first proposed by Dao in 1979,[28] suggests that the pathway by which estrogens are metabolized is an important etiological factor.

Estradiol undergoes initial oxidative conversion to estrone. Estrone and estradiol can undergo hydroxylation by different cytochrome p450 isoenzymes, largely in one of two places: either the 2 or 16 carbon atom. These different metabolites have opposing effects on estrogen receptor-positive breast cancer cells, with 16-hydroxyestrone stimulating proliferation and 2-hydroxyestrone showing no effect on cell growth.[29] The 16-hydroxyestrone form has a roughly ten-fold greater estrogenic activity than 2-hydroxyestrone[30] and has been found to be approximately as genotoxic *in vitro* as 7,12-dimethylbenz[a]anthracene (DMBA), a compound used to initiate breast tumors in animal studies.[31]

The first study to show a correlation between 2:16-hydroxyestradiol ratio and breast cancer risk was published in 1982.[32] The authors compared the serum estradiol metabolites in 33 women with breast cancer to ten normal women. None of the subjects had menstruated in the preceding six months. The serum 16-hydroxyestradiol levels of the breast cancer patients were found to be 50-percent higher than those found in controls; 2-hydroxyestradiol levels were similar in both groups.

Other studies have shown the urinary 2:16-hydroxyestrone ratio to be significantly lower in breast cancer cases compared to controls in premenopausal women,[33] postmenopausal women,[34,35] or both.[36] A recent prospective study found postmenopausal women in the highest tertile of the urinary 2:16-hydroxyestrone ratio were 30-percent less likely to develop breast cancer over follow-up periods of up to 19 years.[37] The optimal urinary ratio in these studies appears to be roughly 2:1, while a 1:1 ratio is associated

with increased cancer risk. Another epidemiological study (n=142) showed no significant correlation between the 2:16-hydroxyestrone ratio and breast cancer risk in postmenopausal women.[38]

Urinary estrogen metabolites have also been proposed as a predictor of cervical cancer risk. One study compared the urinary 2:16-hydroxyestrone ratios of 141 women with cervical intraepithelial neoplasia (CIN) with 132 controls.[39] Significantly lower ratios were seen in women with CIN compared to the controls ($p<0.03$), and the average ratio became progressively lower in women with more severe disease (stages II and III).

Urinary 2:16-hydroxyestrone ratios in men and women with head and neck cancers were compared to age- and sex-matched controls.[40] Low ratios (below 1.0) were found in 30 percent of cases compared with only four percent of controls ($p < 0.05$).

The urinary 2:16-hydroxyestrone ratio may be important in other conditions as well. Both women and men with lupus have been found to have increased concentrations of 16-hydroxylated estrogens in the serum.[41] This finding appeared to be unrelated to medication history. Both 2- and 16-hydroxyestrone have been isolated from human benign breast cysts, although the significance of this finding remains obscure.[42]

Diet and lifestyle factors may be important in determining the ratio of estrogen metabolites. Obese men and women have been found to have low 2:16-hydroxyestrone ratios.[43] Many pesticides have been shown to lower 2:16-hydroxyestrone ratios in animals.[44] Smoking is associated with an increase in 2-hydroxylation of estradiol.[45] The latter finding may at least partially explain the lack of a clear association between smoking and breast cancer risk.

Clinical Trial Data

Several clinical trials have demonstrated the ability of oral supplementation with indole-3-carbinol to increase the 2-hydroxylation of estrogens. A dose-ranging trial found that an oral dose of 300 or 400 mg per day of I-3-C for four weeks was sufficient to significantly increase the urinary 2:16-hydroxyestrone ratio.[46] In another trial, significant increases of urinary 2-hydroxyestrone were seen in five obese women taking 400 mg per day of indole-3-carbinol for two months.[47]

A group of 20 premenopausal women, judged to be at high risk for breast cancer, were supplemented with 400 mg/day I-3-C for three months.[48] Compared to women taking a fiber supplement or placebo, the women taking I-3-C had a statistically significant increase in urinary 2:16-hydroxyestrone ratios. Only three of the 20 women taking I-3-C did not have a clinical response. No adverse effects were noted in this clinical trial.

Patients with recurrent respiratory papillomatosis (n=18), a benign condition of the respiratory tract thought to be associated with human papilloma virus, were supplemented with 200 mg of indole-3-carbinol twice daily (or corresponding pediatric dose).[49] Six patients showed a complete cessation of papilloma growth, and another six patients showed a reduced growth rate. The authors concluded in their preliminary trial that indole-3-carbinol was a safe and efficacious treatment for recurrent respiratory papillomatosis.

The potential for Brassica family vegetables containing dietary indoles to manipulate estrogen metabolism was quantified in a clinical trial.[50] In healthy, postmenopausal women, each 10 g/day increase in Brassica intake led to an increase of 0.08 in the urinary 2:16-hydroxyestrone ratio. The effect of whole foods versus I-3-C has yet to be compared in similar populations.

Epidemiological and Animal Data

Women in the upper decile (10%) of Brassica vegetable intake were found to be at a 40-percent lower risk for breast cancer in a case-control study.[51] Other studies have found little or no protection from Brassica vegetables.[52] A retrospective study with over 1200 participants found that men eating three or more servings of cruciferous vegetables per week were 40-percent less likely to develop prostate cancer than men eating less than one serving per week.[53] Epidemiological studies have also shown increased intake of cruciferous vegetables to be associated with lower risk of lung cancer[54] and non-Hodgkin's lymphoma.[55] One review article reported that 64 percent of published studies found a significant inverse correlation between Brassica vegetable intake and risk of various types of cancer.[56] The most consistent protective effect was seen in lung, stomach, colon, and rectal cancers.

Administration of I-3-C to mice in large doses (34-700 mg/kg/day) has been shown to reduce the incidence of spontaneous mammary tumor formation.[57] Even at these large doses, toxicity was not noted. Large doses of I-3-C (50-100 mg/day) have also been shown to greatly decrease chemical carcinogenesis in rat mammary tissue.[58]

DIM has been shown to have direct treatment effect against mammary cancer in the rat.[9] In this study, rats were fed 5 mg/kg of DIM every other day for twenty days after being treated with a carcinogen. Animals receiving DIM had a four-fold lower tumor burden at the end of the study compared with controls.

I-3-C administration has been shown to prevent cervical cancer in a mouse model.[59] This study used a human papilloma virus to stimulate cervical cancers. I-3-C also appeared to protect against skin cancer and estrogen-mediated fluid retention in the bladder.

The chemopreventive effect of I-3-C has been compared to DIM in only one published animal study to date. In this study, I-3-C and DIM were each found to inhibit chemically-induced carcinogenesis of both the mammary and the forestomach.[26] In each model, I-3-C was more effective than DIM, although not with statistical significance. Oral I-3-C had three times the cytochrome p450 stimulatory activity of DIM in another animal study.[1] While this enzyme stimulation is probably largely responsible for the benefits of I-3-C described above, there may be risks associated with enzyme stimulation as well.

The animal data regarding effect in hepatic cancers initiated by aflatoxin B1 are contradictory. Some studies have shown a preventive effect of dietary I-3-C against hepatic carcinogenesis. When the administration of I-3-C comes after aflatoxin administration, however, a promotion of liver tumors was noted.[60]

I-3-C tumor-promoting activity has also been observed in an animal colon cancer model.[61] When given concurrently with 20-percent beef tallow and one-percent cholesterol, 0.5-percent dietary I-3-C promoted tumors induced by dimethylhydrazine. Another animal study showed no promotion of colon tumor growth.[62] I-3-C was found to enhance promotion of tumors of the thyroid and liver induced by administration of multiple carcinogens.[63]

It is possible that p450 enzyme induction may be producing an increased amount of toxic metabolites of certain chemical carcinogens. In each animal study where there was tumor promotion, a carcinogen was used. Pending further study, the mechanism of this tumor promotion and its applicability to human cancer incidence remains obscure. Enhancing phase II detoxification with antioxidants and other important co-factors may be desirable.

Toxicity

In the four-week dose-ranging study (doses up to 400 mg per day) of indole-3-carbinol supplementation, the only unexplained event noted was a slight elevation of the liver enzyme SGPT within the normal range in two of 57 subjects.[46] No subjects discontinued participation.

In another human study, some adverse effects were noted at higher dosages.[49] One patient taking 400 mg I-3-C twice daily developed imbalance and tremor, which resolved on cutting the dose in half. A two-year-old child accidentally took triple the prescribed dose and developed transient unsteadiness. A 12-year-old who took a triple dose had a transient episode of nausea and unsteadiness. No symptoms persisted beyond discontinuation of medication in any of these patients.

Animal studies have used very high doses of I-3-C without apparent signs of toxicity. Tissue concentrations of over 1 mM (much greater than seen at therapeutic doses) have been safely achieved in these studies.[5]

Animal experiments using DIM in doses up to 5 mg/kg (equivalent to a 350 mg human dose) showed no signs of gross toxicity.[9] To date, no published trials have looked at the safety profile of DIM in humans.

One researcher has referred to I-3-C and DIM as "exodioxins" due to their ability to bind at the aryl hydrocarbon receptor.[18] Dioxin binds at the same receptor, but with a much greater affinity. Therefore, the possibility exists that at very high concentrations dietary indoles could cause the same disastrous p450 enzyme induction seen with dioxin. Epidemiology of cruciferous vegetable intake suggests however, that this is a minimal risk at commonly used dosages of I-3-C.

Conclusion

I-3-C appears to be a safe and promising prevention strategy for breast cancers. The dose required for optimal estrogen metabolite manipulation and minimal side effects has been demonstrated by human trials. While more research needs to be done to elucidate all of the mechanisms of action, I-3-C is ready for large-scale clinical trials in populations judged to be at high risk of developing breast cancer.

Other tumor types may be responsive to I-3-C as well. Prostate, lung, and head and neck cancers are the cancer types with the most animal and *in vitro* support, but other types may be found in the future. Given the direct effect of I-3-C on tumor suppressor genes and oncogenes (including p27, p21, ODC, and NF-kappa-B) there may be a broad applicability of this agent in cancer prevention. Hopefully, future studies will be able to further elucidate the mechanisms behind the tumor promotion seen in certain animal models.

I-3-C may eventually be found to have a role in cancer treatment. The data here are not as strong as for possible cancer prevention, but given the mechanisms of action, the relative safety, and the low expense of I-3-C, it should be assessed as a treatment for active cancers. Also, its additive action with tamoxifen makes it a potential choice in hormone-receptor positive breast cancers treated with hormonal blockade.

Future studies will need to compare the safety and efficacy of I-3-C with some of its major metabolites, including DIM, ICZ, and LTr-1. To date, no peer-reviewed human studies have looked at the safety or recommended dose of any of these agents. Given that the effects of I-3-C are likely due to downstream metabolites, the possibility exists that one of these agents may be found to be more efficacious. On the other hand, the *in vitro* evidence suggests that each of the metabolites may have an important role in the action of I-3-C. At this time, there is insufficient evidence to assume that administration of a single I-3-C metabolite would have a more beneficial effect *in vivo*.

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