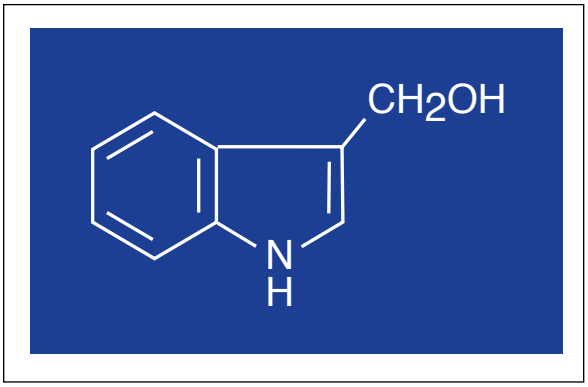

Indole-3-Carbinol

Introduction

Indole-3-carbinol (I3C) is a compound found in high concentrations in Brassica family vegetables, including broccoli, cauliflower, Brussels sprouts, and cabbage. As a nutritional supplement, I3C has received attention in recent years as a promising preventive and treatment agent for breast and other types of cancers, and may have beneficial effect in the management of *Herpes simplex* virus (HSV) and human papilloma virus (HPV). Preliminary studies have examined its efficacy, safety, and optimal dosage. Early studies have been promising, and will likely be followed by larger clinical trials.

Pharmacokinetics

Animal studies have shown intravenous or intraperitoneal administration of I3C does not have the effects seen with oral dosing,[1] suggesting other metabolic products are at least partially responsible for the action of I3C. After ingestion, I3C undergoes dimerization in stomach acid. The majority of ingested I3C is absorbed in the small intestine as the dimer, diindolylmethane (DIM). Several other metabolites are formed as well, including 2-(indol-3-ylmethyl)-3,3'diindolylmethane (LTr-1, a trimer, also referred to as BII) and indolylcarbazole (ICZ).

Following oral administration of 400 mg I3C to humans, serum levels of DIM reached 0.1-0.4 mcg/mL.[2] Serum I3C was not measurable at this dosage. Presence or absence of other products, such as LTr-1 or ICZ, was not reported. An animal study noted the formation of DIM and LTr-1 are pH dependent, with optimal production at a pH between 4 and 5.[3]

Animal studies have shown DIM and LTr-1 are the major serum metabolites following oral administration of I3C.[3] Although other metabolites are measurable in smaller amounts on high performance liquid chromatography, DIM and LTr-1 levels are roughly equivalent, with different metabolites being more prevalent in different organs. Another study quantified hepatic concentrations of I3C metabolites, finding 24-percent DIM, 20-percent LTr-1, 24-percent of another metabolite called 1-(3-hydroxymethyl)-indolyl3-indolylmethane, and a number of minor metabolites.[4] The metabolite ICZ was found in very small concentrations.

Initially, the major route of elimination of I3C metabolites is urinary. After 40 hours of continuous dietary administration, however, the fecal route becomes prevalent.[4] I3C metabolites demonstrated a serum half-life greater than 48 hours after one week of continuous administration in animal studies.[4]

Mechanism of Action

Indole-3-carbinol has a number of potential mechanisms of action that are associated with chemoprevention of cancer. While many mechanisms have been described, it is possible others exist, particularly among the minor I3C metabolites that have not been well studied.

Several I3C metabolites have anti-estrogenic activity. Both I3C[5] and ICZ[6] compete with estrogen for binding sites. DIM has been shown to selectively bind to estrogen receptors, and may act as an estrogen antagonist at physiological concentrations.[7]

Other research has focused on the ability of I3C metabolites, specifically DIM and ICZ, to induce the cytochrome p450 isoenzymes CYP1A1 and CYP1A2.[3,8] Through its action on these enzymes, I3C alters the pathway of estrogen metabolism in human males and females in a manner that decreases the risk of certain tumors.[9-11] Metabolism is altered by inducing specific cytochrome p450 isoforms, via the aryl hydrocarbon receptor,[12] for which DIM is a weak ligand.[13] Metabolic degradation of estradiol by hepatocytes results primarily in either 2-hydroxyestrone or 16α-hydroxyestrone, and to a lesser extent 4-hydroxyestrone, a potent carcinogen. It is known that 16α-hydroxyestrone causes proliferation of some breast tumor cell lines,[14] while the alternative metabolite, 2-hydroxyestrone, demonstrates anti-estrogenic and anti-proliferative activity.[15]

I3C reduces the activity of the tumor-promoting enzymes ornithine decarboxylase (ODC) and tyrosine kinase at *in vitro* concentrations of 250-1,000 μM.[16] Indole-3-carbinol increased p21 and p27 expression in MCF-7 breast cancer cells.[17] It is currently unclear whether these effects on enzymes and genes are significant at concentrations of I3C achievable in the serum of humans, although ODC inhibition has been observed in animal studies.[18]

I3C has been shown to inhibit cell cycle progression at the G1 checkpoint and elevate p53 tumor suppression levels in MCF10A human breast cancer cells.[19] Further laboratory studies have demonstrated I3C to induce apoptosis in various cell types, including myeloid, leukemia and breast cancer cells, through inactivation of the nuclear factor-kappaB (NF-κB) pathway.[20-22] It is thought that this mechanism may provide the molecular basis for I3C to suppress overall tumorigenesis.

Clinical Indications

Breast Cancer

The first study to show a correlation between 2:16-hydroxyestradiol ratio and breast cancer risk was published in 1982.[23] The authors compared the serum estradiol metabolites in 33 women with breast cancer to 10 healthy 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,[24] postmenopausal women,[25,26] or both.[27] Another 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.[28] 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.[29]

A 12-week phase I trial was conducted on 17 women (1 postmenopausal and 16 premenopausal) from a high-risk breast cancer cohort, to determine tolerability and effects of I3C supplementation on the 2:16-hydroxyestrone ratio.[30] Subjects were on placebo for four weeks, then four weeks of 400 mg I3C daily, followed by four weeks of 800 mg I3C daily. Indole-3-carbinol dosing increased CYP1A2 activity in 94 percent of participants, a mean increase of 410 percent, which was echoed by a 66-percent rise in the urinary 2:16-hydroxyestrone ratio with administration of I3C. Maximum increase of this ratio was observed at 400 mg per day, with no additional benefit at 800 mg daily.

Several studies have examined the effects of I3C and its metabolites on breast cancer cell lines. I3C and tamoxifen have been shown to act separately and/or cooperatively to inhibit the growth of estrogen receptor-positive (ER+) breast cancer cells.[31] I3C has also been shown *in vitro* to inhibit the ability of human breast cancer cells to invade surrounding tissue.[32] This effect is mediated through up-regulation of tumor suppressor gene PTEN and adhesion molecule E-cadherin.

DIM inhibited proliferation of human breast cancer cells at concentrations achievable through oral supplementation with I3C (10-50 μM).[13] Incubation of human breast cancer cells with DIM has been found to stimulate p53-independent apoptosis.[33] Another laboratory study demonstrated that DIM can induce apoptosis in breast cancer cells independent of

estrogen receptor status by influencing the Bcl-2 family-mediated apoptotic regulatory factors.[34]

LTr-1 inhibited the growth of estrogen receptor-positive and -negative breast cancer cells *in vitro*.[35] This metabolite has estrogen-antagonizing activity that may be at least partially responsible for the effect in ER+ lines.

Prostate Cancer

Several laboratory studies have observed I3C to induce apoptosis in human prostate cancer cells. One study demonstrated I3C led to cell cycle arrest at the G1 checkpoint in human prostate cancer cell lines at concentrations from 25-100 μM.[36] I3C appeared to induce expression of the p21 and p27 tumor suppressor genes, and down-regulate NF-κB. In another study, I3C induced G1 cell cycle arrest and inhibited prostate specific antigen (PSA) synthesis in LNCaP human prostate cancer cell lines by inhibiting cyclin-dependent kinase (CDK) activity and stimulating synthesis of the p16 CDK inhibitor.[37] I3C and sulforaphane (another constituent of cruciferous vegetables) significantly inhibited the proliferation of PC-3 prostate cancer cells *in vitro* at concentrations of 0.2 mM/L and 0.02 mM/L, respectively.[38]

In one study, DIM was found to both suppress cell proliferation of LNCaP prostate cancer cells and inhibit dihydrotestosterone (DHT) stimulation of DNA synthesis.[39] It was observed that DIM acts as a competitive inhibitor of DHT binding to androgen receptor sites.

Colon Cancer

In one laboratory study I3C significantly reduced colon cancer cell proliferation at concentrations of > 0.1mM.[40]

Cervical Cancer/Cervical Dysplasia/ HPV

Urinary estrogen metabolites have been proposed as a predictor of cervical cancer risk. Women with cervical intraepithelial neoplasia II/III (CIN II/III) have lower 2:16-hydroxyestrone ratios than women with no abnormal cervical pathology;[41] therefore, the goal is to up-regulate 2-hydroxylation, resulting in metabolism of estradiol to a benign product at the expense of 16α-hydroxylation. Among several compounds proven to be effective in up-regulating 2-hydroxylation, I3C was found to be the most potent.[42] Indole-3-carbinol also appears to suppress 4-hydroxylation activity.[43]

In a double-blind, placebo-controlled study, 30 patients with biopsy-confirmed CIN II/III were randomized to receive placebo, 200 mg, or 400 mg oral I3C daily for 12 weeks.[44] Three patients failed to complete the study. None of the 10 patients in the placebo group experienced complete regression of CIN. In contrast, four of eight patients in the 200-mg/day group and four of nine in the 400-mg/day group demonstrated complete regression of CIN, based on 12-week biopsy. In this study, the highest dose, 400 mg/day, is equivalent to the amount found in one-third of a head of cabbage. No adverse effects were noted in this or previous studies.

An *in vitro* study of I3C was performed on human cervical cancer cell lines, concluding both I3C and DIM were able to achieve apoptosis in the cervical epithelium of HPV-16 transgenic mice and suggesting its use as a potential chemotherapeutic agent.[45] I3C has been shown *in vitro* to block the estrogenic stimulation of HPV expression.[46] HPV is implicated in the pathogenesis of cervical, head, and neck cancers.

Recurrent Respiratory Papillomatosis

Patients with recurrent respiratory papillomatosis (n=33), 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 over a period of five years.[47] Eleven patients had a complete cessation of papilloma growth, and another 10 patients showed a reduced growth rate; no immediate long-term effects were found. The authors concluded that indole-3-carbinol was a safe and efficacious treatment for recurrent respiratory papillomatosis.

Herpes simplex Virus

One study reported indole-3-carbinol may inhibit replication of HSV by interfering with CDK.[48] Cyclin-dependent kinases are essential for replication of the virus, as they are required for the transcription of HSV genes. According to researchers, I3C may

deny HSV the cell cycle factors needed for replication, by somehow disrupting the function of CDK p21 or p53. Results from RNA isolation and gene expression studies corroborate this mechanism. According to the report, I3C completely inhibited HSV replication in tissue culture. Further human studies are warranted; however, this finding may serve as a harbinger for the use of I3C in the treatment of HSV infection.

Drug-Nutrient Interactions

Because I3C has anti-estrogenic activity, practitioners might wonder if it would be synergistic with drugs like tamoxifen. I3C and tamoxifen appeared to function by different mechanisms; I3C can stimulate apoptosis in estrogen receptor-negative human breast cancer cell lines as well.[49]

Side Effects and Toxicity

In general, I3C has been well tolerated by individuals at dosage ranges between 400 and 800 mg daily. A recent phase I trial of I3C in 17 women at high risk for breast cancer noted doses up to 800 mg daily were well tolerated in all participants.[27]

However, at doses of 800 mg or higher, there has been one report of three episodes where some adverse effect from I3C was noted.[50] One patient taking 400 mg I3C twice daily developed imbalance and tremor, which resolved on cutting the dose by 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 I3C without apparent signs of toxicity. Tissue concentrations over 1 mM (much greater than seen at therapeutic doses) have been safely achieved in these studies.[4]

Dosage

Typical dosage for I3C is in the range of 200-400 mg daily. Several clinical trials have demonstrated the ability to significantly increase urinary 2:16-hydroxyestrone ratio at doses of 300-400 mg daily in as little as four weeks.[30,51]

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