**INDOLE-3-CARBINOL**
Muhammed Majeed, Ph.D and Lakshmi Prakash, Ph.D.
Sabinsa Corporation, New Jersey, U.S.A.

**Introduction:**

Indole-3-carbinol (I3C) is a compound found in *Brassica oleracea* (cruciferous) vegetables, such as cabbage, brussels sprouts and broccoli. These vegetables have been researched since the mid-nineteenth century on account of their physiological effects on humans and ruminants. Two types of sulfur containing compounds are present in all cruciferous vegetables: the glucosinolates (formerly called thioglucosides) and S-methyl cysteine sulfoxide. I3C is a metabolite of the glucosinolate, glucobrassicin. In recent years, this compound has been shown to inhibit human breast and ovarian cancers.

Estrogen has been implicated in the etiology of breast and ovarian cancers. A metabolite of estrogen, 16α-hydroxyestrone has been found to have a significant role in the development of viral, carcinogen induced and oncogene-transfected tumors. I3C is thought to inhibit the conversion of estrogen to this compound during hydroxylation by promoting an alternative pathway that produces 2-hydroxyestrone. The second compound is a weak anti-estrogen and has no tumorigenic effects. In effect, I3C inhibits the formation of the "carcinogenic" form of estrogen metabolite, simultaneously depleting the available estrogen pool for its formation, thereby facilitating chemoprevention.

**Chemistry:**

I3C is chemically 3-indole methanol.

![Chemical structure of I3C](image)
It’s acid derived condensation product, Indole-[3,2 β]-carbazole (I3Z) has been shown to exhibit anti-estrogenic action. The relative contributions of I3C and I3Z in chemoprevention were studied using trout microinjection assay. In these studies, DNA binding with a known carcinogen, AFB1 (Aflatoxin B1) was studied in the presence of the inhibitory compounds. The results revealed that the acid condensation products, not the parent compound represent the anticarcinogenic species in trout and that their formation in the stomach is a prerequisite for I3C anticarcinogenesis\(^3\). I3C is also substantially converted into its dimer, 3,3’ diindolylmethane Indole-3-carbinol and related congeners appear to protect partly via radical and electrophile scavenging\(^4\). They are therefore powerful antioxidants that prevent the progression of free radical induced deteriorative changes in the body.

**Biological effects:**

A. **Preclinical studies**
1. Early studies on laboratory animals revealed that the chemopreventive effect of I3C in chemically induced carcinogenesis was through modulation of the oxidative metabolism of carcinogens in the liver\(^5\)\(^6\)\(^7\). These reactions are mediated through the cytochrome P-450 mixed function oxidases. I3C therefore positively influences the action of these enzymes.
2. I3C has been proven to be an anti-initiator of carcinogenesis, functioning as a chemoprotectant in mice and trout, if administered before a carcinogen\(^8\)\(^9\). However, there was no protective effect in trout if the compound was administered after carcinogen exposure\(^10\).
3. I3C incorporated in the diet of C3H/OuJ mice was found to produce a significant reduction in spontaneous induction of mammary tumors\(^11\). The authors of this study found that both tumor incidence and generation of multiple tumors were reduced by I3C in a dose dependent manner, in mice exposed to murine mammary tumor virus. They concluded that I3C is effective at the
promotional phase in murine tumorigenesis. Additionally, these researchers reported that high doses of I3C (2000 ppm) did not produce toxic effects in mice.

4. Antitumor effects in mammary carcinogenesis induced in Sprague-Dawley rats fed diets rich in brussels sprouts have also been reported\textsuperscript{12}. The mechanism of tumor inhibition has been postulated\textsuperscript{11}.

In estrogen metabolism, estradiol is oxidized by the enzyme 17β-estradiol dehydrogenase to estrone. Estrone is then hydroxylated by specific enzymes and converted to either 16α-hydroxyestrone or 2-hydroxyestrone. 16α-hydroxyestrone formation has been associated with tumorigenesis. The C-2 hydroxylase which produces 2-hydroxyestrone is a microsomal enzyme associated with cytochrome P-450A1 and 1A2. I3C has been shown to enhance C-2 hydroxylation in human volunteers\textsuperscript{13}. According to earlier reports, however, intestinal enzyme activity was increased six to 20 fold in response to 50-500 ppm of I3C while the hepatic enzymes remained unchanged\textsuperscript{14}. The authors of a more recent study\textsuperscript{11} concluded that the mechanism of tumor inhibition by I3C through induction of the enzyme cytochrome P-450A1, which in turn favors the C-2 hydroxylase pathway, is specific to estrogen responsive cells and found only at high doses of 500 ppm. Human breast cancer cells therefore selectively respond to I3C. This study validates the efficacy of I3C in the chemoprevention of mammary tumorigenesis.

\textbf{B. Clinical studies:}

An early clinical trial established that C-2 hydroxylation is facilitated by cytochrome P-4501A1 and enhanced in human volunteers fed I3C\textsuperscript{13}. A recent clinical study\textsuperscript{15} tested the hypothesis that the estrogen metabolite ratio 2-OH-estrone: estriol can be raised via dietary indole-3-carbinol (I3C) and that this higher ratio could be sustained over a 3-month test period. Using a randomized clinical trial with three arms, each containing 20 subjects, arm 1 received 400 mg/day of I3C daily for 3 months, arm 2 received 20 g of alpha-cellulose daily for the same time period as a source of added fiber, and arm 3 received a
placebo dose. Blood levels of a variety of biochemical parameters were measured. The urinary 2-OH-estrone: estriol estrogen metabolite ratio was measured monthly at the same time of the menstrual cycle. No changes were observed in the control and alpha-cellulose-treated arms. A substantial mean increase in the ratio was observed in the I3C-treated arm at month 1 and the increase was maintained over the 3-month time in that period. Three of the 20 subjects in this I3C-treated group differed from the others in that no significant change in the metabolite ratio was observed at any time point. The results suggest that I3C can serve to increase the 2-OH-estrone:estriol metabolite ratio in a sustained manner without detectable side effects although some individuals may be resistant to such change.

Thus, Indole-3-carbinol is a readily available phytochemical with proven clinical utility in the prevention of breast cancer and other hormone-dependent cancers.

References:
4. Shertzer HG.et al.. (1996) Molecular modeling parameters predict antioxidant efficacy of 3-indolyl compounds. Archives of Toxicology.70(12):830-4


