Breast Cancer and Dietary Indole 3 carbinol – A review

Priyanka Surushe*1 Mayuri Bari1, Rahul Hajare1, Anil Chandewar1 Swapnil Surushe2

1. P. Wadhawani college of Pharmacy, Yavatmal 445001, Maharashtra, India.
2. Anuradha College of Pharmacy, Chikhali, Maharashtra, India.

Corresponding Author Email- priyusurushe2@gmail.com

Abstract
Estrogens are known for their proliferative effects on estrogen-sensitive tissues resulting in tumorigenesis cause Breast cancer. Metabolism of Estrogen by Cytochrome p-450 produces metabolites 2-hydroxyestrone (2-OH E1), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestrone (4-OH E1), 4-hydroxyestradiol (4-OHE2) and 16α-hydroxyestrone (16α-OHE1). The C-16-alpha version tends to damage DNA and cause abnormal cellular proliferation, while the C-2 metabolite has less estrogenic activity. Indole 3 carbinol (I3C) is a naturally occurring compound derived from cruciferous vegetables such as broccoli, Brussels sprouts, and cabbage that actively promotes the breakdown of estrogen to the beneficial metabolite, 2-OH. I-3-C undergoes dimerization in strong acid, like that normally present in gastric fluid. Several products may be formed, but the majority of ingested I-3-C is absorbed in the small intestine as the dimer, Diindolylmethane (DIM). Both I-3-C and DIM inhibit tumorigenesis. I-3-C favours the C2-metabolic pathway and inhibits C4 and C16 metabolic pathways.

Keywords- Breast cancer, Indole 3 carbinol, Cytochrome p-450, cruciferous, Diindolylmethane.

Introduction
Estrogens exert diverse biological effects in animals and humans, and many of these effects result from a direct interaction of the estrogen with an intracellular receptor that activates the expression of genes encoding proteins with important biological functions (1–4). One of the most important and notable effects of estrogens is a super potent mitogenic action in hormone sensitive tissues such as the uterus (5, 6) and breast (7–9). Prolonged exposure of target tissues or cells to excessive mitogenic stimulation by natural or synthetic estrogens has long been considered an important etiological factor for the induction of estrogen-associated cancers in experimental animals (10, 11) and humans (10,12–18). Estrogenic hormones are eliminated from the body by metabolic conversion to hormonally inactive (or less active) water-soluble metabolites that are excreted in the urine and/or feces. The metabolic disposition of estrogens includes oxidative metabolism (largely hydroxylations; reviewed in ref. 19) and conjugative metabolism by glucuronidation (20–22), sulfonation (23–27) and/or O-methylation (28–30).

Members of the cytochrome P450 family are the major enzymes catalyzing β- nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH*)-dependent oxidative metabolism of estrogens to multiple hydroxylated metabolites. Although most of the oxidative metabolism of estrogens takes place in liver, some estrogen-metabolizing isoforms of the cytochromes P450 that are usually expressed at low or undetectable levels in liver are selectively expressed in certain extra hepatic tissues. Indole-3-carbinol, a compound that occurs naturally in vegetables such as cabbage, induces the expression of cytochrome P-450 1A1, which shifts the estrogen metabolic pathway in favor of C-2 hydroxylation and away from the formation of 16 hydroxyestrone, a suspected endogenous carcinogen. Increased 16-hydroxylation of estrogen is associated with greater risk of breast cancer.
The production of 4-hydroxyestrone is also inhibited by I3C. I3C can also induce a G1 cell-cycle arrest of human MCF-7 breast cancer cells that is accompanied by the selective inhibition of cyclin-dependent kinase 6 expression and stimulation of p21 gene expression. In indole-treated cells, a fraction of I3C was converted into natural Diindole product -3,3'-diindolylmethane, which accumulates in the nucleus; suggests that Diindollmethane (DIM) may have a role in the transcriptional activities of I3C. Indole-3-carbinol from Brassica vegetables also contains micronutrients that provide additional DNA protection from reactive oxygen species the growth of breast cancer cells. Oxidative stress is believed to have a complex and multifunctional role in the development of most age related chronic diseases. In the context of carcinogenesis, increased oxidative stress may damage DNA beyond DNA repair capacity, leading to the clonal expansion of initiated cells.

Metabolic Fate of Estrogens

Normal premenopausal women produce several hundred micrograms of estradiol (E2) daily. A portion of the roughly 1017 newly synthesized molecules find their way to binding sites in the nucleus and other organelles of many tissues. Once bound to estrogen receptors, the estrogen hormones elicit an increased rate of DNA synthesis, resulting in gene transcription and cell division. Meanwhile, a similar number of estrone/estradiol molecules are removed from the body pool, maintaining a relatively constant stimulation of cell division in estrogen-sensitive tissues. Much of the estradiol is converted into estrone (E1) and estriol (E3), but these are only two of the best known metabolites. The half-life of E2 is about three hours. [31] Its removal is accomplished by irreversible conversion into metabolites that may be passed into urine or bile. There are multiple pathways that convert E2 to products that have widely different biological activities. Some products are powerful carcinogens while others act as estrogen antagonists. The relative amounts of these metabolites control the overall cancer risk from estrogen exposure.

Estrogens are metabolized by a series of oxidizing enzymes in the cytochrome P450 family. These are the detoxification enzymes that break down all manner of drugs, hormones, and environmental toxins into generally less harmful metabolites. , scientists have discovered how the parent estrogen compounds are modified in the C-2, C-4, or C-16 pathways Oxidation to form hydroxy derivatives is a principal route of endogenous steroid metabolism (Figure 1). Particular enzymes within this family, namely cytochrome P450 1A1 and 1A2, are activated or stimulated and then more parent estrogens are metabolized into C-2-hydroxylated compounds. The iso-enzyme that catalyzes 2-hydroxylation of E2 (Cytochrome- CYP1A1) is an inducible enzyme. It is formed in greater amounts in hepatic microsomes in response to dietary ingredients and cigarette smoke. A separate enzyme, CYP1B1, catalyzes 16α-hydroxylation. This enzyme is not inducible by diet, but xenobiotic carcinogens and pesticides may stimulate its activity. [32] As a result, a preferential increase in 2-hydroxylation can occur through dietary manipulation. After estrone hydroxylation, the various poly hydroxy derivatives are conjugated with glucuronate or sulfate, or methylation occurs prior to excretion in urine. The catechol-O-methyl transferase (COMT) enzymes that catalyze the methylation reactions require S-adenosyl methionine.

A portion of conjugated and unconjugated steroids also passes into bile, some of which may be reabsorbed via enterohepatic circulation. Lower intestinal reuptake rates can explain why total estrogen loads are decreased by high fiber diets and especially by the lignans contained in flax seed. The principal hydroxylation products are 2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestrone (4-OHE1), 4- hydroxyestradiol (4-OHE2), and 16α-hydroxyestrone (16α-OHE1). (Figure no.1) The 2-hydroxy derivatives and 16α-OHE1 have opposite biological properties. Cell proliferative activity of the 2-hydroxy metabolites is nil, while 16α-OHE1 is a powerful estrogen agonist. The C-16-alpha version tends to damage DNA and cause abnormal cellular proliferation, while the C-2 metabolite has less estrogenic activity[33,34] If the proportion of C-16-alpha-hydroxyestrone can be decreased while the C-2-hydroxyestrone is increased—changing the ratio between the
two—cancer risk could be reduced[35-38] . The 4-hydroxy derivatives are also estrogen agonists, but their relative concentrations are smaller, so they may have less impact on cancer risk compared to the more abundant 2- and 16α-derivatives. The carcinogenic affects of 4-OHE1 may be due to the effects of toxic quinone metabolites rather than to estrogen agonist effects. [39]

Note that the 16α-derivative of estrone is the precursor to relatively inactive estriol. Apparently, it is the unique orientation of the 16α-OH group with the keto group of estrone that leads to the potent effects of this metabolite.[40] Sixteen-α-OHE1 can bind covalently to sites in the endoplasmic reticulum while becoming simultaneously bound to nuclear estrogen receptor sites.[41] This binding stimulates heightened activity for days instead of hours. The 16α-OHE1 effects persist until the binding proteins are degraded. Such increased cell proliferative and genotoxic effects appear to be a mechanism of cancer induction by tumor viruses, carcinogens, and oncogenes associated with breast cancer.[42] Early evidence suggested that 2- OHE1, at most, behaved as a weak estrogen and probably more as an anti-estrogen in several models.[43,44] Cell culture studies in subsequent years also identified 2-OHE1 as a weak promotional estrogen that was less potent than 16α- OHE1 at initiating cell proliferation.[45] Comparison of the relative potencies of 2-OHE1 and 16α- OHE1 at transforming cells showed 16α-OHE1 exhibited increased unscheduled DNA synthesis, proliferation, and anchorage-independent growth relative to 2-OHE1, which showed less activity than estradiol in each of these parameters.[46,47] In long-term proliferation studies, persistent proliferation was observed after treatment of human ER positive cancer cells with 16α-OHE1 but not with 2-OHE1.[48]
enzymatic hydrolysis, releases indole-3-carbinol (I-3-C). I-3-C undergoes dimerization in strong acid, like that normally present in gastric fluid. Several products may be formed, but the majority of ingested I-3-C is absorbed in the small intestine as the dimer, Diindollmethane (DIM) (Figure 2). Both I-3-C and DIM inhibit tumor genesis. The use of I-3-C as a novel approach to breast cancer prevention was reviewed in 1995. [49] Hepatic CYP450 levels in the rat exhibit dose-response relationship to I-3-C. [49] The enzyme activities showed linear increases from 10 to 150 mol/ mg protein.min with oral doses ranging from 1 to 500 mol/animal. When compared with other dietary indoles, I-3-C is the most potent inducer of 2-hydroxylation enzymes. Associations of I-3-C with reduced incidence of tumors induced by dimethylbenzanthracene, aromatic hydrocarbons, or other free radical generators were the first signs of positive effects against cancer. [50, 51, 52] Numerous lines of evidence support the conclusion that I-3-C induces hepatic CYP4501A1 with the resultant increase in 2 hydroxyestrogens. [53-58] Several groups have explored the question of I-3-C’s impact on cell proliferative and neoplastic events. Data from animal models, [59-62] human cell cultures [63-68] and human population cancer incidence, [69-71] have been examined. Evidence for actions of I-3-C separate from that of DIM comes from studies of cell proliferation in vitro. [72] A type of cell that represents premalignant keratinocytes showed abnormal proliferation when exposed to E2 or 16\(\alpha\)-OHE1. The proliferation was blocked by I-3-C. I-3-C induces cell cycle arrest in breast cancer cells through inhibition of cyclin-dependent gene expression. So, although DIM appears to be the active I-3-C metabolite required for increased 2-hydroxylation, there may be other specific cell-regulating effects of I-3-C or its other metabolites not exhibited by DIM. Evidence linking cigarette smoking with increased 2-OHE formation adds support for use of I-3-C and Diindollmethane (DIM) to reduce risk of hormone-dependent tumors by a similar mechanism. Female smokers have increased hepatic estrogen 2-hydroxylation, a finding that may account for the anti-estrogenic effects of cigarette smoking. [73] The same metabolic effect is seen in men. [74]

**Figure no.2 Structure. A- Indole 3 carbinol (I 3 C), B- Diindolylmethane (DIM).**

Some of the components of cigarette smoke induce CYP1A1. Responses to I-3-C vary among individuals due to genetic polymorphic variation in the inducibility of CYP1A1. The effect is easily seen in comparisons between Caucasian and African- American women. Baseline differences in the 2/16\(\alpha\) ratio were almost two-fold higher for Caucasian women. [75] However, there were significant increases in 2/16 ratios in all subjects after ingestion of I-3-C, and African- American women had higher percentage increases than Caucasian women. [76] Women of both groups who carry Msp1 polymorphism in its heterozygous form respond much less to I-3-C. This observation means that women should be monitored to see if the ratio is increasing before continuing I-3-C supplementation. One specific dietary regimen that has been reported to be effective is a high fiber diet including the consumption of 50 g of cabbage or 100 g of broccoli twice weekly. [77]

**Conclusion-**

Approximately one-third of all cancer cases are related to dietary influences, and several lines of evidence indicate a female’s diet during adolescence can affect her health during her mid-thirties. Whether a given woman has other
risk factors such as viral exposures or enzyme induction resistance, awareness of estrogen metabolism tendencies can be an important part of overall evaluation of cancer risk. The incidence of cancer of the breast and other estrogen-sensitive tissue is increased by exposure to estrogen and especially by increased 16α-OHE1 in the presence of low conversion to 2-OHE1. The 2/16α ratio is the most modifiable and possibly the single greatest factor impacting estrogen-sensitive cancer risk. The effects of 16α-OHE1 elevations begin as soon as a female begins to secrete high levels of estrogens, so the earlier dietary interventions are begun, the greater the risk reduction. Indole 3 carbinol as phytoconstituent in cruciferous vegetables such as broccoli, Brussels sprouts, and cabbage in daily diet interfere with estrogen metabolism, which shifts the estrogen metabolic pathway in favor of C-2 hydroxylation and away from the formation of 16 hydroxyestrone, a suspected endogenous carcinogen. So Indole 3 carbinol is important phytoconstituent in daily diet to reduce the risk of estrogen induced tumarogenesis which results in Breast cancer.

References-


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