THE ROLE OF DIINDOLYL METHANE IN THE PREVENTION AND TREATMENT OF CANCER

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ABSTRACT

Cancer is a major public health burden in both developed and developing countries. It was estimated that there were 10.9 million new cases, 6.7 million deaths. Although there are several treatment approaches for cancer, major drawback of these systems is the toxicity which may even lead to death. Development of Non-toxic drugs which can be useful in cancer treatment is the most important need of the hour. Of the 92 anticancer drugs commercially available prior to 1983 in the US and among worldwide approved anticancer drugs between 1983 and 1994, 60% are of natural origin. Improved cytotoxic agents continue to be an important line in the discovery of modern anticancer drugs. Herbal drugs have been used in the treatment and prevention of various diseases including cancer. Diindolylmethane (DIM), a phytochemical. Phytochemicals is generally referred to the compounds exclusive of essential nutrients that have specific biological activity to human. It has been known that over 10,000 different phytochemicals possess the potential preventive or supplementary effect on various diseases. From several decades ago to now, cancer continues to be the leading-lethal cause worldwide. Studies have shown that natural phytochemicals derived from certain plants have the capability to prevent carcinogenesis. Diindolylmethane (DIM), a phytochemical found in cruciferous vegetables such as cabbage, Brussels sprouts, broccoli, and kale, appears to cause infected cells to die. Cervical cancer is a common cancer of the female reproductive system, specifically the cervix of the uterus. Cervical cancer can be fatal if left untreated. The good news is that cervical is highly preventable and treatable if caught in its early stage. DIM could cause cell death of both human cervical cancer cells and the HPV-16-infected cervical cells of mice. Another promising study was done on a form of the phytochemical called indole-3-carbinol.
(I3C), which is released when you chew or crush a cruciferous vegetable and is immediately converted to DIM. Most research has focused on this phytochemical's ability to normalize estrogen metabolism, since estrogen plays a role in cervical cancer.

**Key words:** Phytochemical, DIM, Biological activity, Carcinogens, Nutrient.

**OBJECTIVE:** The objective of this review is to emphasize the role of DIM prevention and treatment of cancer.

**INTRODUCTION**

The declaration of the "war on cancer," research on carcinogenesis has led to the realization that cancer is not a single disease. Cancer is, in fact, a biomedically complex group of diseases resulting partly from changes in genes that control cell growth and behavior and partly from interactions between these genetic changes and the cellular stresses from specific environmental and behavioral factors, including lifestyle choices such as diet. Cancer is a growing health problem around the world particularly with the steady rise in life expectancy, increasing urbanization and the subsequent changes in environmental conditions, including lifestyle. According to a recent report by the World Health Organization (WHO), there are now more than 10 million cases of cancer per year worldwide. Although there is no 'magic bullet' that can completely conquer cancer, many types of the disease might be avoidable. Cancer risk can be reduced by eliminating the identified carcinogens—or at least minimizing exposure to them. Reduction of cancer risk by either preventing carcinogenesis or stopping carcinogenesis in its early stages is a logical approach for reducing the cancer burden, both for high-risk individuals and for the general population. Prevention is the ultimate approach to controlling cancer. Diindolylmethane is a natural substance formed during the breakdown of glucobrassicin present in food plants of the Brassica genus. The Brassica genus includes cabbage, broccoli, Brussels sprouts, cauliflower and kale. Brassica vegetables via the mother compound glucobrassicin.[1][2][3] As glucobrassicin degrades into I3C by the plant-contained enzyme Myrosinase, deactivation of this enzyme by heat-treatment (cooking) can reduce the oral bioavailability of any glucosinolate including DIM.[4][5] Some bioavailability is retained, however, due to human intestines expressing Myrosinase as well.[6] Ingested glucobrassicin is catalyzed via the enzyme Myrosinase (stored in vegetables) and turns into Indole-3-Carbinol, which is rapidly digested into both DIM and various other metabolites in the human stomach via acid-mediated condensation reactions.[7][8]. It has potent effects on estrogen metabolism and is able to keep the body
relatively balanced (by preventing either drastic increases or decreases in estrogen). In small amounts, it can both inhibit the aromatase enzyme (and prevent conversion of testosterone into estrogen) and it can act on more potent forms of estrogen and convert them into less potent forms; this conversion reduces the overall effects of estrogen in the body. However, taking too much DIM at once can actually induce the aromatase enzyme and act in the opposite manner and increase estrogen synthesis. DIM also exerts numerous anti-carcinogenic (anti-cancer) effects in the body and is one of the reasons this vegetable family is seen as healthy.

Structure of DIM

Sources

- Brussel Sprouts
- Mustard Greens, chopped)
- Turnip,
- Savoy Cabbage,
- Kale
- Watercress
- Kohlrabi
- Red Cabbage
- Broccoli
- Cauliflower
Efficacious use of DIM
DIM has been tested in humans. The entire basis of absorbable DIM's introduction as a supplement was based on testing which supported its safe and efficacious use. Many of these studies involve side-by-side testing of DIM and I3C. Recent publications have begun to focus on DIM, recognizing it as more advantageous than I3C. DIM has been shown to be more potent and active than I3C in experimental cervical cancer [9] When inducing cell death (apoptosis) in cervical cancer cells, Dr. Karen Auborn, a renowned cancer research scientist, states that "DIM is a more effective inducer of apoptosis than I3C. Other work showed that DIM had a strong anti-proliferative effect in human endometrial cancer cells Indolocarbazole (ICZ), the dioxin-like and enzyme-inducing reaction product arising from I3C, notably failed to control the growth of these endometrial cancer cells[10 ] Unwanted enzyme-inducing effects from I3C have also been shown in recent animal studies[11 ]This work clearly demonstrates that oral I3C can increase production of dangerous estrogen metabolites at the same time it increases the beneficial 2-hydroxy metabolites. In effect, this cancels out benefits for estrogen metabolism. Consistent with this, I3C has been shown to be ineffective in controlling the growth of experimental breast cancer [12,13] while DIM succeeds[14,15].

Use of absorbable DIM has been shown effective in amounts close to that obtainable from our diet (0.3 mg/kg/day of DIM) [16] I3C requires about 15 times more than this (4.5 mg/kg/day[17]) and is associated with side effects [18,19]. In preventive nutrition, even small risks and potential toxicities must be taken seriously, lest the negatives outweigh the positives and cause harm. DIM is complexed with Vitamin-E TPGS to provide for and enhance DIM's absorption. Vitamin-E TPGS (Tocophersolan) is a well-known ingredient in foods and pharmaceuticals. It is so safe as to appear on the Generally Regarded As Safe (GRAS) list maintained for ingredients by the FDA. Rarely are dietary supplement ingredients safe enough to appear on this list. Proof of its safety has been established in extensive testing, including testing by the NIH [20]. Vitamin-E TPGS is also used to improve the absorption of Vitamin-D [21] and Vitamin-E [22] in infants.

Epidermal Growth Factor Receptor (EGFR) mutants are associated with resistance to chemotherapy, radiation, and targeted therapies. Here we found that the phytochemical 3,3′-Diindolylmethane (DIM) can inhibit the growth and also the invasion of breast cancer, glioma, and non-small cell lung cancer cells regardless of which EGFR mutant is expressed and the drug-resistant phenotype. DIM reduced an array of growth factor signaling pathways
and altered cell cycle regulators and apoptotic proteins favoring cell cycle arrest and apoptosis. Therefore, DIM may be used in treatment regimens to inhibit cancer cell growth and invasion, and potentially overcome EGFR mutant-associated drug resistance[23].

**Molecular Targets of DIM**

DIM has been shown to active nuclear factor kappa-beta (nF-kB) signalling, caspase activation, cytochrome P450 activation (specifically CYP1A1, CYP1A2, and CYP19), DNA repair, the aryl hydrocarbon receptor (AHR) and various protein kinases.[24, 25]

**Interactions of DIM with Hormones Estrogen & Testosterone**

'Estrogen' is a term used to refer to a class of molecules with similar activities (sort of like how 'Androgen' is a blanket statement for many molecules), and DIM can cause a shift in estrogen ratios to cause less estrogenic effects DIM appears to be an estrogen regulatory compound, as it possesses both pro-estrogenic and anti-estrogenic mechanisms of action. In a pro-estrogenic sense, DIM is known as an estrogen receptor beta agonist, and exerts these effects through non-ligand (binding) means, theorized to be through protein kinases.[26]. In the liver, activation of the Aryl Hydrocarbon receptor (AhR) is an area of focus when looking at estrogenic effects of DIM. Binding to the Ah receptor (in general) can cause more aromatase to be synthesized and cause greater conversion of testosterone to estrogen, and DIM can bind to the Ah receptor as well as directly induce the aromatase enzyme causing it to increase [27]. However, its binding to the AhR is weak, and coingestion of DIM alongside PCBs or Dioxins (common industrial estrogenic compounds) causes less of a spike in estrogen than isolated PCBs or Dioxins, and relatively less estrogenic activity[28,29]When looking at the enzymes of P450 and liver detoxification, DIM appears to inherently be pro-estrogenic but may act as an antagonist in the presence of stronger estrogens and thus cause a relative decline. Beyond liver enzyme interactions, the modification of estrogen metabolites also produces an ultimate state in which more of the 2-hydroxyestrogens relative to 16a-hydroxyestrogens and 4-hydroxyestrogens results in less overall estrogenic actions in vivo.[30] This is due to induction (activation) of the estradiol-2-hydroxylase enzyme, an enzyme that converts estrogens and estrones to their 2-hydroxylated form which is seen as chemoprotective[30] and in some cases anti-estrogenic while not influencing 4 and 16a-hydroxylation, two metabolites which are genotoxic and retain estrogenic properties.[31] In regards to androgen metabolism, DIM appears to be a strong antagonist in human prostate cancer cells[32].
DIM Inhibits Human Papilloma Virus (HPV) Growth

3,3'-Diindolylmethane is a dietary indole from cruciferous vegetables that has demonstrated pre-clinical therapeutic efficacy in models of DMBA-induced mammary cancer, transplanted human breast cancer, and in models of human papilloma virus (HPV) related disease. Animal and human use of crystalline diindolylmethane has revealed the need for absorption-enhancing technology to allow adequate gastro-intestinal uptake. BioResponse-DIM, a patented formulation of diindolylmethane categorized and sold as a dietary supplement, utilizes solubility-enhancing micro-encapsulation technology to allow absorption of effective amounts of diindolylmethane. Human use of this formulation promotes a dose-responsive upward effect on the urinary ratio of 2-OH/16-OH estrone metabolites, demonstrated by ELISA testing of urine before and after use. In previous prospective studies, a greater 2-OH/16-OH estrone urinary ratio has been associated with a lowered risk of future breast cancer. We are able to monitor compliance by measurement of urinary diindolylmethane using gas chromatography-mass spectrometry. Human use of this preparation at higher doses has demonstrated treatment-related resolution of moderate and severe cervical dysplasia in preliminary open-label testing. A still higher dose, about 10 times above that possible from dietary exposure to diindolylmethane from vegetable sources, has resulted in the control of laryngeal papillomas and resolution of cutaneous and plantar warts in preliminary human testing. The clearing of HPV-related lesions is consistent with diindolylmethane's previously described, apoptosis-promoting and chemopreventive activity[33].

Indole-3-carbinol (I3C) and its dimer 3,3'-diindolylmethane (DIM), obtained from dietary consumption of cruciferous vegetables, have multiple biochemical activities. Both compounds have been effective clinically in treating precancerous lesions of the cervix and laryngeal papillomas, pathologies with a human papillomavirus (HPV) component. Using cDNA microarrays, we examined early changes in gene expression after treatment with 100 micro mol/L DIM in C33A and CaSki cervical cancer cells and in an immortalized human epithelial cell line (HaCat), as well as in normal human foreskin keratinocytes (HFK). Multiple analyses were done after treating C33A cells for 6 h; other analyses included 4- and 12-h treatments of C33A and 6-h treatments of CaSki, HaCat and HFK cells. DIM consistently altered the expression of >100 genes at least twofold. Many of the stimulated genes encode transcription factors and proteins involved in signaling, stress response and growth.

DIM not only suppressed transcription of a luciferase gene driven by the HPV11 upstream regulatory region (URR) in C33A, CaSki, HaCat and HFK cells from >2-fold to 37-fold
depending on the type of cells, but also reduced endogenous transcription of HPV16 oncogenes to undetectable levels in CaSki cells as determined by an RNase protection assay. Ectopic expression of GADD153 or NF-IL6 suppressed transcription in a dose-dependent manner driven by the HPV11 URR in C33A, CaSki, HaCat and HFK cells. These results identify unexpected ways in which dietary I3C and DIM invoke cellular responses and are consistent with a potential antiviral effect of DIM on keratinocytes, but they do not explain the differential sensitivity [34]. Dietary indole-3-carbinol (I3C) has clinical benefits for both cervical cancer and laryngeal papillomatosis, and causes apoptosis of breast cancer cells in vitro. We asked whether I3C and its major acid-catalyzed condensation product diindolylmethane (DIM), which is produced in the stomach after consumption of cruciferous vegetables, could induce apoptosis of cervical cancer cell lines. We also asked whether this effect could be observed in vivo. In vitro, both I3C and DIM caused accumulation of DNA strand breaks in three cervical cancer cell lines. Induction of apoptosis was confirmed by nuclear morphology, nucleosome leakage, altered cytoplasmic membrane permeability and caspase 3 activation. Neither I3C nor DIM caused apoptotic changes in normal human keratinocytes. In C33A cervical cancer cells, DIM was more potent than I3C [dose at which the number of viable cells was 50% of that in untreated cultures (LD(50)) = 50-60 micromol/L for DIM and 200 micromol/L for I3C in a mitochondrial function assay] and faster acting. Furthermore, I3C reduced Bcl-2 protein in a time- and dose-dependent manner. In HPV16-transgenic mice, which develop cervical cancer after chronic estradiol exposure, apoptotic cells were detected in cervical epithelium by TdT-mediated dUTP nick-end labeling staining and by immunohistochemical staining of active caspase 3 only in mice exposed to 17beta-estradiol (E2) and fed I3C. Rare apoptotic cells were also observed by hematoxylin and eosin staining in the spinous layer of the cervical epithelium in both control and transgenic mice. Estradiol reduced the percentage of these late-stage apoptotic cells in the cervical epithelium of transgenic, E2-treated mice, but this reduction was prevented by I3C[35].

CONCLUSION
DIM has a consistently positive safety record which makes a strong argument for its safe, effective and long-term use in humans. For those interested in a scientifically-based, cruciferous supplement for estrogen-related concerns, DIM provides distinct advantages over I3C. DIM is a conservative, well-studied, safe, and clearly effective natural substance. Epidemiological studies have shown that a diet rich in fruits and cruciferous vegetables is
associated with a lower risk of cancer. Indole-3-carbinol (I3C) and its dimeric product 3,3'-diindolylmethane (DIM) have been shown to exhibit anti-tumor activity both in vitro and in vivo. 3,3'-Diindolylmethane is a compound derived from the digestion of indole-3-carbinol, found in Brassica vegetables. It has anti cancer benefits in lab studies but, as of May 2010, no human studies with this supplement could be found in terms of its use in prevention or treatment of various forms of cancer.

REFERENCES
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