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The Anti-Carcinogenic Effect of Indole-3-Carbinol and 3, 3'-Diindolylmethane and their Mechanism of Action

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Abstract

Indoles are aromatic heterocyclic organic compound and became the precursor to many pharmaceuticals. Indoles are found naturally in cruciferous plants and indole derivatives, indole-3-carbinole (I3C) and 3, 3'-diindolylmethane (DIM) can also be synthesized by a variety of methods. *In vitro* studies indicated that I3C and DIM inhibit cell proliferation, caused cell cycle arrest at G1 phase and induced apoptosis. The precise molecular mechanisms by which indole derivatives exert their tumor suppressive effects in human cancer cells are still unclear. It was reported that indoles alter estrogen metabolism. Microarray gene expression profiling and other studies indicated that indoles regulate many genes that are important for the control of cell cycle, cell proliferation, apoptosis, signal transduction, angiogenesis and cell invasion. In addition, it was found that indoles prevent tumor formation of breast and prostate cancer in animal models. Furthermore, these derivatives were evaluated in human clinical trials phase I and phase II as a potential chemopreventive agents against human breast, ovary, and vulvar intraepithelial neoplasia and colon cancers. Preliminary findings of these studies showed a significant clinical improvement. Interestingly, the use of indole derivatives was found to be safe without any indicated side effects. In conclusion, the results provide an evidence of the benefit of indole-derivatives in the prevention and treatment of hormone-dependent and hormone-independent human cancer. Further clinical trials are needed in order to approve the efficacy of indole derivatives in treatment of human cancer and to evaluate the indole use by the Food and Drug Administration (FDA).

Keywords: Glucosinolates; Indole-3-carbinol; 3,3'-diindolylmethane; Cancer treatment; Cancer prevention

Abbreviations: DIM: 3:3'-Diindolylmethane; I3C: Indole-3-Carbinol; CDK6: Cyclin-Dependent Kinase 6; NF-$_k$B: Nuclear Factor-$_k$B; ROS: Reactive Oxygen Species; PI3-K/Akt: Phosphatidylinositol 3-Kinase; JNK: C-Jun NH(2)-Terminal Kinase; DMBA: Dimethylbenz(A)Anthracene; BP: Benzo(A)Pyrene; DEN: Diethylnitrosamine; ER: Endoplasmic Reticulum; CYP: Cytochrome P450; DHT: Dihydrotestosterone; PARP: Poly(ADP-Ribose) Polymerase; TFDP: Transcription Factor Dp; CBFB: Core Binding Factor Beta; STI6: Suppressor of Tumorigenecity; EGFR: Epidermal Growth Factor Receptor; AR: Androgen Receptor; HPV: Human Papilloma Virus; TRAIL: Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

Introduction

Epidemiological studies show that increased consumption of cruciferous vegetables such as cabbage, brussels sprouts, broccoli and cauliflower, is associated with decreased incidence of human cancer. The protective effect of cruciferous vegetables includes colon cancer [1-3], gastric cancer [4], breast [5-7], thyroid [8], mesothelioma [9] and prostatic cancer [10]. In addition to the epidemiological data, experimental animal studies have been carried out in order to confirm the protective effect of cruciferous vegetables in retarding the development of chemically induced human hepatic or mammary tumorigenesis [11-15].

The anticarcinogenic effects of indole-diet derivatives in experimental animal models and in humans raise the special attention of these compounds as possible chemopreventive agents. Here we summarized the data regarding the inhibitory effects of indole derivatives on human cancer and provide the possible molecular mechanisms of these compounds in the treatment and prevention of human cancer.

Indole Compounds

Cruciferous vegetables contain large amounts of glucosinolates, such as glucobrassicin and neoglucobrassicin [16,17]. Glucobrassicin in cruciferous vegetables undergoes hydrolysis by the enzyme myrosinase, and the main product of this hydrolysis is 3-indolylmethyl glucobrassicin. When a vegetable is cut or chewed, 3-indolylmethyl glucobrassicin is hydrolyzed to form indole-3-carbinol (I3C) [18] (Figure 1). In an acidic environment and low pH, I3C may condense into other polymeric products were 3,3'-diindolylmethane (DIM) and ascorbigene are the main products (Figure 2). In order to study the *in vivo* disposition of I3C, rainbow trout was fed with radiolabeled [5-^{3}H]-indole-3-carbinol. After 48 hours, 40% of total radioactivity was found in the liver extracts as DIM [19]. When I3C was incubated in a simulated gastric condition, it was found that the reaction mixture contained over 20 products were DIM was found to be about 10-20% and I3C was found only 0.5% of total products [20]. *In vivo* studies using rats showed that DIM was detected in gastric contents, stomach tissue, small intestine and liver after one hour of receiving oral dose of I3C [21].

The Anti-Cancer Effect of Indole-Diet Derivatives

The effect of indole derivatives on breast cancer

Breast cancer is the most common malignancy diagnosed in women in Western countries. It is well known that elevated levels of estradiol-17 β activity and presence of a functional estrogen receptor

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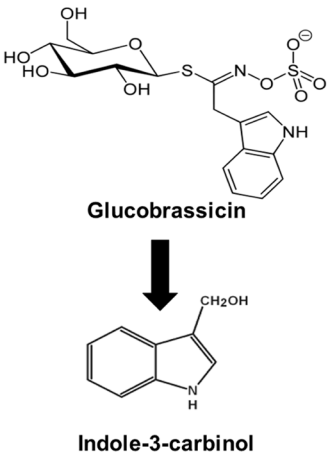

Figure 1: Molecular structure and metabolism of I3C.

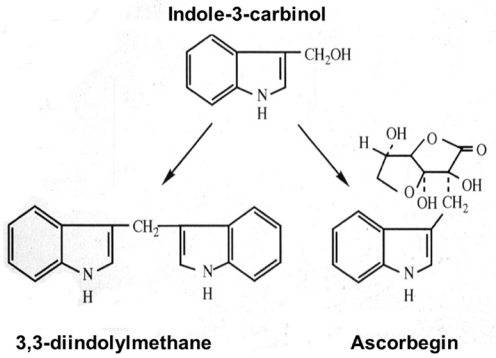

Figure 2: The chemical structure of Indole-3-carbinol, 3,3'-diindolylmethane and ascorbigen.

in human breast tissue increased the risk of breast cancer [22-24]. Reduction in breast cancer risk was often associated with consumption of carrots and green vegetables that are rich in carotenoids. Experimental studies indicated that feeding mice with dried cabbage decreased incidence of pulmonary metastases after intravenous injection with mammary tumor cells [25]. Moreover, it was found that consumption of cruciferous vegetables increases the ratio of 2-hydroxyestrone to 16-α-hydroxyestrone, the oxidative product of 16-α-hydroxyestradiol-17β, and reduces the incidence of breast cancer in women [26,27]. Experimental studies *in vitro* indicated that I3C, DIM as well as cabbage juices affect the expression of CYP1 family genes encoding the key enzymes of estrogen synthesis [28,29]. Other studies indicated that indole derivatives are significantly reduced the expression of estrogen alpha-receptor in human breast cancer cells [30-32]. Several studies have examined the effects on I3C and its metabolites on growth of human cancer cells lines including breast [33-37]. I3C and DIM suppressed the growth of human breast cancer cells of estrogen receptor positive and estrogen receptor negative. In the molecular mechanism it was found that I3C inhibited cyclin-dependent kinase 6 (CDK6), induced p27 expression and reduced the level of retinoblastoma protein. Other studies indicated that I3C also induced cell cycle arrest in breast cancer cells and inhibited CdK6, but in high concentration of 200 μM [37-39]. In addition, it was demonstrated that I3C activates p53 phosphorylation and disruption of the p53-MDM2 interaction, which releases p53 to induce the p21 CDK inhibition and G1 cell cycle arrest [30,40]. These studies suggested that I3C induced phosphorylation of phosphatidyl-inositol-3-kinase (PI3-K/Akt) family member, ataxia telangiectasia gene that activates p53 through its phosphorelation that induce $p21^{WAF1}$ CDK inhibitor and caused a G1 cell cycle arrest. Moreover, studies from different laboratories have shown that indole derivatives are potent inducers of apoptosis in breast cancer cells [34-43]. DIM induced apoptosis in human breast cancer cells through inactivation of PI3-K/Akt and nuclear factor-kB ($NF\text{-}_kB$) pathways [43]. PI3-K/Akt signaling pathway is an important signal transduction pathway in cells and plays a critical role in controlling cell survival and apoptosis. It was reported that PI3-K/Akt regulates the $NF\text{-}_kB$ activation directly through activation of IkB kinase (IKK) or phosphorylation of RelA [44]. On the other hand, $NF\text{-}_kB$ is a key regulator of genes involved in cell activation and proliferation [45-47]. Therefore, $NF\text{-}_kB$ has been described as a major therapeutic target in cancer where inhibition of its activities generally believed to suppress tumorigenesis and the progression of tumors. Furthermore, it was shown that I3C induced BRCA1 and BRCA2 expression and that both I3C and BRCA1 inhibit estrogen-stimulated estrogen receptor alpha activity in human breast cancer [48]. BRCA1 and BRCA2 have been identified as tumor suppressors for several different hormone responsive cancer types including breast cancer. BRCA1 expression is decreased or absent in a significant proportion of sporadic breast cancer because of hypermethylation of the gene promoter [49,50].

Other studies indicated that I3C down regulated epidermal growth factor receptor (EGFR) in human breast cancer cell lines and induce cell cycle arrest and apoptosis [51,52]. In addition, it was shown that DIM treatment induced hyperpolarization of mitochondrial inner membrane, decreased cellular ATP level, and significantly stimulated mitochondrial reactive oxygen species (ROS) production. ROS production, in turn, led to the activation of stress-activated pathways involving p38 and c-Jun NH(2)-terminal kinase (JNK). Using specific kinase inhibitors (SB203580 and SP600125), it was shown that the central role of p38 and JNK in cells where DIM-induced $p21^{WAF1}$, is in the transcription level. In addition, antioxidants significantly attenuated DIM-induced activation of p38 and JNK and induction of p21, indicating that oxidative stress is the major trigger of these events [53].

The role of indole-diet derivatives in breast cancer prevention and treatment was examined in animal models. Mice fed with dried cabbage showed a decreased incidence of pulmonary metastases after intravenous injection of mammary tumor cells [54] and reduced the incidence of spontaneous estrogen-responsive tumors such as mammary [55]. On the other hand, diet containing cabbage significantly lower incidence of mammary cancer induced by N-methyl-N-nitrosourea in female Sprague-Dawley rats [15]. In addition, it was reported that the oral administration of female Sprague-Dawley rats with DIM or I3C before administration of the carcinogens; dimethylbenz(a)anthracene (DMBA) that induced mammary tumor formation or benzo(a)pyrene (BP) that induced neoplasia of the forestomach, significantly inhibited tumor formation [56,57]. Other study in our laboratory indicated that ascorbigen was found to have the ability to inhibit the formation of DMBA-initiated mammary tumors in female Sprague-Dawley rats (Unpublished data).

However, *in vitro* studies using human breast cancer cells suggest possible modes of indole derivatives activity. However, little is known regarding the *in vivo* mechanism of action where indoles inhibit mammary tumor growth. One interesting possibility that have been received attention is the ability of DIM to inhibit angiogenesis. The results indicated that DIM strongly inhibited proliferation, migration,

invasion and capillary tube formation in cultured human umbilical vein endothelial cells. In a complementary *in vivo* Matrigel plug angiogenesis assay it was observed that treatment of 5 mg/kg of DIM inhibited neovascularization by 76% compared to untreated animals. In addition this dose of DIM also inhibited the growth of human MCF-7 cell tumor xenografts by up to 64% in female athymic mice. Interestingly, these studies did not observe any weight loss or toxicity to major organs of the animal [57,58]. Other study indicated that I3C suppressed the capillary-like tube formation by phorbol myristate acetate-stimulated endothelial EA hy926 cells and such inhibition was associated with decreased vascular endothelial growth factor and its receptor [59]. In addition, extracts of broccoli suppressed 12-O-tetradecanoylphorbol-13-acetate-induced cancer cell invasion and matrix metallopropeinase-9 activity and therefore suppressed the invasiveness of human breast cancer cells *in vitro* [60].

The effect of indole derivatives on prostate cancer

Prostate cancer is the most common diagnosed malignancy (accounting for 29% of the newly diagnosed cancers) and the second leading cause of male death in Western industrialized countries [61]. Age, race, and family history are known risk factors for this disease. However, recently it has been found that nutritional and hormonal risk factors are also involved in prostate cancer [62]. Mortality from prostate cancer results from metastases to the bones and lymph nodes and progression from androgen-dependent to androgen-independent prostatic growth [63]. Androgen withdrawal causes involution of the prostate gland, as a result of inhibition of cellular proliferation and stimulation of apoptosis of the androgen-dependent cells. Although androgen withdrawal remains the only effective therapy for men with advanced disease, in approximately 80% of the patients, progression to the lethal and untreatable stage of androgen-independence eventually occurs. The increasing incidence of prostate cancer in men all over the world, has led to the performance of intense investigations, searching for compounds having efficient anti-carcinogenic effects against this type of cancer. These studies examined mainly apoptotic properties of the compounds investigated on human prostate cancer cells, under *in vitro* and *in vivo* conditions. The results indicated that I3C and DIM suppress the growth of human prostate cancer cells in a dose- and time-dependent manner [64-70]. DIM exhibited potent antiproliferative and antiandrogenic properties in androgen-dependent human prostate cancer cells. As a part of the antiproliferative mechanism of I3C in human prostate cancer, it was found that I3C represses the expression of androgen receptor [71,72]. DIM suppresses cell proliferation of androgen-dependent, cells, LNCaP, and inhibits dihydrotestosterone (DHT) stimulation of DNA synthesis. These effects were not found in androgen-independent PC-3 cells. Interestingly, results of receptor binding assays indicated that DIM is a strong competitive inhibitor of DHT binding to androgen receptor [72]. Moreover, DIM inhibited endogenous PSA transcription and reduced intracellular and secreted PSA protein levels induced by DHT in LNCaP cells [73]. In addition, the growth inhibition of prostate cancer cells by I3C or DIM found to be through cell-cycle arrest at G1 checkpoint [64,74-76]. I3C inhibited the expression of CDK6 and upregulated the expression of $p21^{WAF1}$ and $p27^{K1P1}$ in well differentiated (LNCaP) and poorly differentiated (PC3) cells suggesting the inhibition of cell-cycle progression [77]. Furthermore, I3C and DIM induced apoptosis in human prostate cancer cells. We provided evidence that the induction of apoptosis in human prostate cancer cells by DIM is exerted through the mitochondrial pathway [78]. DIM triggers cytochrome C translocation from the mitochondria to the cytosol that promotes the activation of caspase 9 that activate effector caspases; 3 and 6 in a time-dependent manner. The activation of these effectors leads to the cleavage of poly (ADP-ribose) polymarase (PARP), an enzyme implicated in DNA damage and repair mechanisms. Other studies indicated that I3C decreases the expression of Bcl-2 and Bcl_{XL} genes that inhibit apoptosis, and upregulated Bax gene which induced apoptosis [65,78]. Translocation of Bax from cytosol to the mitochondria, causing the mitochondrial permeability transition, loss of mitochondrial potential, release of cytochrome C, activation of caspases followed by cleavage of PARP and inducing DNA fragmentation and thus inducing apoptosis [78]. It was reported that I3C significantly inhibited NF-$_k$B DNA binding in PC3 cells and thus inhibition of cell proliferation together with induction of apoptosis [64,79]. In addition, it was found that I3C and DIM downregulated the expression of Pol II transcription factor Dp (TFDP), the nuclear factor YC (NF-YC) and the core binding factor beta (CBFB) that play an important role in transcription, cell-cycle progression and oncogenesis [80]. On the other hand, these derivatives upregulated the expression of the suppressor tumorigenicity 16 (ST16) [81]. It was reported also that indole derivatives inhibited epidermal growth factor receptor (EGFR) expression, PI3K/Akt activation and abrogated the EGF-induced activation of PI3K/Akt in prostate cancer cells [82-84]. PI3K/Akt signal transduction pathway plays a critical role in controlling the balance between cell survival and apoptosis in human cancer cells [84]. Other study indicated that I3C increased the expression of two tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptors (DR4 and DR5). Treatment of LNCaP cells with I3C induced DR4 and DR5 expression at both transcriptional and translational levels [85]. It was also reported that DIM could activate the AMPK signaling pathway, associated with suppression of the mammalian target of rapamycin (mTOR), down-regulation of androgen receptor (AR) expression, and induction of apoptosis in both androgen-sensitive LNCaP and androgen-insensitive C4-2B prostate cancer cells. DIM also activates AMP-protein kinase (AMPK) and down-regulates AR in androgen-independent C4-2B prostate tumor xenografts in SCID mice. These results suggest that DIM could be used as a potential anti-cancer agent in the clinic for prevention and/or treatment of prostate cancer regardless of androgen responsiveness, although functional AR may be required [86].

The global gene expression profiles of prostate cancer cells following I3C or DIM treatment were not clear. To obtain comprehensive gene expression profile of indole derivatives in human prostate cancer, cDNA microarray was utilized. A total of 738 genes that showed a greater than two fold change were detected after 24 h of treatment with DIM. Among these genes, 677 genes were down regulated and 61 were upregulated [47]. It was concluded that I3C and DIM affected the expression of a large number of genes that are related to the control of carcinogenesis, cell survival and physiologic behaviors. These results may lead to determine the molecular mechanism by which indole derivatives exert their anticarinogenic effect in human prostate cancer cells.

The *in vivo* activity of DIM was investigated by us using male C57BL/6 mice in which tumors were initiated by subcutaneous transplantation of TRAMP-C2 cells derived from a primary tumor of the C57BL/6 prostate. The results indicated that treatment with DIM (2.5 mg, 5 mg or 10 mg/kg) 3 times a week for 3 weeks, significantly diminished tumor growth. Histological examination of tumor sections revealed that DIM decreased cell proliferation as it was detected by the anti-mouse Ki-67 staining. Ki-67 is a nuclear protein expressed during all active phases of cell cycle. Moreover, it was found that DIM induced apoptosis in the tumor cells as it was detected by DAPI staining. DAPI is a fluorescence compound that specifically binds to DNA and allows visualization of its morphology. Treatment with DIM had no effect

on body weight or kidney and liver functioning [77]. Other study examined the effect of I3C on prostate cancer model using Copenhagen rats and the transplantable cell line, MAT-LyLu, a cell line derived from a prostate tumor of Copenhagen rats and it is androgen independent and metastasizes to the lung and lymph nodes. The results indicated that I3C inhibited the incidence, growth and metastases of MAT-LyLu cells [76]. Using the same animal model it was found that fed male Copenhagen rats with diets containing 10% broccoli, decreased tumor weight by 42%. Tumor growth reduction was associated with reduced proliferation and increased apoptosis [87]. Moreover, treatment of mice with DIM inhibits tumor formation of TRAMP cells, mouse prostate cancer cells [88].

These results indicated that indolic compounds, I3C and DIM, may constitute important anti-carcinogenic and therapeutic food derivatives against human prostate cancer offering natural compounds with minimal toxic effects in the treatment and prevention of human prostate cancer.

The effect of indole derivatives on other types of cancer

Dietary indoles has been documented as inhibiting tumorigenesis in various target organs including colon [89-91], thyroid [92], pancreas [93], liver [94], cervix [95,96], melanoma [97], and lung [98,99].

In vitro studies indicated that DIM substantially decreased the number of viable cells and induced apoptosis of the human colorectal cancer cell lines; HCT116, well differentiated with p53 wild-type, and HT-29, moderately differentiated with p53 mutant, in a concentration-dependent manner [100]. This effect was mediated through the increase of the translocation of cytochrome C from the mitochondria to the cytoplasm. This was followed by the activation of caspases and the activation of PARP cleavage without affecting the protein levels of p53, Bax or Fas ligand but it decreased the protein level of Bcl-2. Other study indicated that DIM and I3C induce apoptosis in human colorectal cancer cells; LS-174, Caco-2 and HCEC. It was also demonstrates that DIM stimulates activating transcription factor 3 (ATF3) expression through ATF4-mediated pathway and subsequently induces apoptosis in human colon cancer cells [101]. In experimental animal model it was shown that cruciferous vegetables inhibited chemically induced colon cancer [102].

The anti-thyroid cancer activity of I3C and DIM were studied using different thyroid cancer cell lines representative of papillary (B-CPAP and 8505-C cells) and Follicular (CGTH-W-1 and ML-1) carcinoma of the thyroid. These studies indicated that DIM is more potent than I3C in inhibiting cell survival of both papillary and follicular thyroid carcinoma cells. The anti-proliferative effect of DIM was mediated by G1 arrest followed by induction of apoptosis. Interestingly, DIM also inhibited the growth of primary thyroid goiter cells by 70% [91]. These studies provide a strong rational for the clinical exploration of DIM as an adjuvant to surgery in thyroid proliferation diseases.

Human papilloma virus (HPV) is an important risk factor for cervical cancer [103]. Transgenic mice that express cancer-promoting HPV genes develop cervical cancer following treatment with 17β-estradiol. Using this model it was shown that feeding the transgenic animals with I3C significantly reduced the number of mice that developed cervical cancer [104].

The hepatic tumor-modulatory properties of I3C fed to C57BL/6 mice initiated with diethylnitrosamine (DEN) were studied. The results indicated that long-term administration of I3C in the diet inhibits DEN-initiated hepatocarcinogenesis in the infant mouse model [93].

Because I3C was known to induce estradiol 2-hydroxylase and reduce estrogen activity, the possible inhibiting effect of I3C on spontaneous occurrence of endometrial adenocarcinoma in female Donryn rats was examined. In this strain of rats, the related estrogen/progesterone ratio is increasing with age and the high incidence of endometrial cancer is detected. The results indicated that the incidences of endometrial adenocarcinoma in rats fed I3C were significantly smaller than that detected in the control group. These results suggested that dietary I3C inhibits spontaneous occurrence of endometrial adenocarcinoma, an effect that may be due to the induction of estradiol 2-hydroxylation [105].

DIM and its derivatives inhibited the growth and induce apoptosis of human pancreatic cells *in vitro*. The decrease in cell proliferation was accompanied by increased indoplasmic reticulum (ER) staining and calcium release. This was accompanied by increased expression of glucose related protein 78 and C/EBP homologous transcription factor (CHOP/GADD153) proteins showing induction of ER stress leads to activation of the apoptotic pathway [92]. This study indicated also that DIM increase the expression of the TRAIL death receptor, DR5. Therefore, two molecular mechanistic pathways of DIM were suggested in human pancreatic cancer cells; activation DR5, receptor-dependent pathway, and inducing ER stress, receptor independent pathway. DNA microarray studies using transformed keratinocytes and tumor cell lines suggested that cellular mRNA content from genes associated with ER stress response (GADD153, ATF3, EBP-β) was increases within 4-6 hours of exposure to pharmaceutical concentrations of DIM [106]. Other studies using human breast and prostate cancer cell lines support the hypothesis that cytotoxic concentrations of DIM can activate cellular stress response pathways *in vitro* including the ER stress response. Other major stress-associated genes were also upregulated by DIM, such as; GADD153, GADD45A, ATF4, GADD34, GRP78, GRP94' XBP-1 and asparagines synthase [107]. It was also reported that I3C exhibited the highest potency in radical scavenging activity and was most protective against oxidative stress in neuronal cell assays [108].

Human Clinical Studies

The use of vegetables for hundreds of years, the hundreds of carefully observed animal studies, and the consumption of synthetic I3C by thousands of people without noticeable harmful effects have shown that I3C is a safe dietary supplement. Therefore, Indole-diet derivatives were found to be safe compounds and they were evaluated in human clinical trials as potential chemopreventive agents. In phase I trial of I3C, 17 women from a high-risk breast cancer were used [109]. The results indicated that daily administration of I3C at doses of 400 and 800 mg was well tolerated by all subjects. These dose levels produced significant changes in the activities of at least two xenobiotic and steroid-metabolizing enzymes and markedly altered the ratio of hydroxylated estrone metabolites in a manner consistent with chemoprevention. The use of 400 mg of I3C daily seems to be safe and effective supplement for women [106,107,109,110]. Other study indicated that DIM supplementation at a dose of 108 mg/day for 30 days increased urinary 2-hydroxyestrone excretions in postmenopausal women with a history of breast cancer [110].

Other report of phase II study determined the potential therapeutic benefits of I3C in the management of vulvar intraepithelial neoplasia [111]. Preliminary findings of this study showed a significant clinical improvement in symptomatology and vulvoscopic appearance of vulvar intraepithelial neoplasia with I3C therapy.

The effect of I3C on the progression of cervical cancer in women was examined. It was found that four of eight women, who took 200 mg/day of I3C for 12 weeks, had complete regression of the tumor, while none of the ten who took a placebo had complete regression [112].

The therapeutic benefits of indole-3-carbinol (I3C) in the management of vulvar intraepithelial neoplasia (VIN) was reported. Women with histologically confirmed high-grade VIN were randomized to receive 200 and 400 mg/day of I3C. This study has shown significant clinical improvement in symptomatology and vulvoscopic appearance of VIN with I3C therapy. Further clinical and scientific investigations are required to support these preliminary findings [111].

These results are similar to that found in animal studies where I3C and DIM exhibited a profound chemopreventive effect. Although the preliminary results seem to be promising, large controlled clinical trials are needed in order to determine the efficacy of the indole derivatives on the preventing the progression of human cancer.

Safety

Sixty women at increased risk for breast cancer were enrolled in a placebo-controlled, double blind dose-ranging chemoprevention study of I3C. Fifty-seven of these women with a mean age of 47 years range [21-72] completed the study. Each woman took a placebo capsule or an I3C capsule daily for a total of 4 weeks. None of the women experienced any significant toxicity effects. However slight increases in the serum concentrations of a liver enzyme (alanine aminotransferase; ALT) were observed in two women who took unspecified doses of I3C supplements for four weeks [113]. Other study indicated that one person reported a skin rash while taking 375 mg/day of I3C [114]. On the other hand high doses of I3C (800 mg/day) were associated with symptoms of disequilibrium and tremor, which resolved when the dose was decreased [115].

No drug interactions in humans have been reported. However, preliminary evidence that I3C and DIM can increase the activity of CYP1A2 [116,117] suggests the potential for I3C or DIM supplementation to decrease serum concentrations of medications metabolized by CYP1A2. Both I3C and DIM modestly increase the activity of CYP3A4 in rats when administered chronically [66]. This observation raises the potential for adverse drug interactions between in humans since CYP3A4 is involved in the metabolism of approximately 60% of therapeutic drugs.

The safety of I3C or DIM supplements during pregnancy or lactation has not been established.

Conclusions

These studies prove evidence that the diet-derived indole derivatives, I3C and DIM, exert anticancer effects mediated through the regulation of cell cycle, induction of apoptosis, transcription, cell signal transduction, inhibiting angiogenesis and suppressing cell invasion (Figure 3). The activation of the mitochondrial pathway through releasing of cytochrome C and activation of caspases, together with inactivation of hormonal, PI3K/Akt, MAPK, Bcl-2 and NF-$_k$B pathways may represent the possible molecular mechanism of indole-derivatives in their anticancer activity. The anti-cancer activity of I3C and DIM was detected in various target organs including breast, prostate, colon, liver, cervix, endometrium, melanoma and lung using human cancer cell lines or various animal models. Furthermore, these derivatives were evaluated in clinical trials phase I and phase II as a potential chemopreventive agents against breast, ovary and colon cancers. Preliminary findings of these studies showed a significant clinical improvement. In addition, the use of indole derivatives was found to be safe without any indicated side effects.

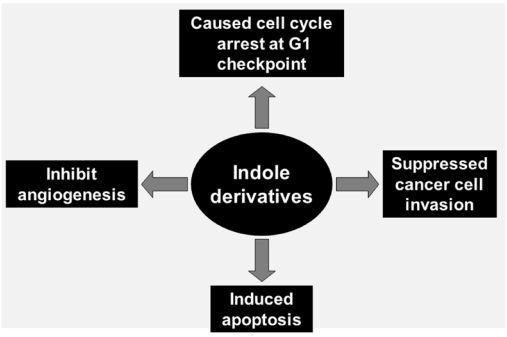

Figure 3: Activities assigned to indole-diet derivatives.

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