

Review

Indole-3-carbinol as a Chemoprotective Agent in Breast and Prostate Cancer

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Abstract. Assessment of the oral use of indole-3-carbinol (I3C) as a chemoprotective compound has not sufficiently considered the chemical instability of I3C. This review addresses the question of whether I3C is directly active in its own right or only serves as a precursor, with all of the biological responses coming from reaction products arising in culture media and in the presence of stomach acid. Because of the rapid conversion of I3C into its dimer, diindolylmethane (DIM), and trimers very little circulating I3C is present following oral use to effect a biological response. Reports of toxicity associated with oral use of I3C relate to unfavorable enzyme induction, which can be attributed to non-DIM reaction products. Because DIM provides a predictable, safer response than the mélange of compounds derived from I3C DIM should be regarded as the chemoprotective compound of choice.

In studies going back to early work by Wattenberg, it was shown that compounds present in cruciferous vegetables had potent anticancer activity (1). In crucifers, active phytochemicals are derived from precursor glucosinolates in plant cells through the action of myrosinase enzymes. Chemical analysis showed that the activity could be accounted for by two groups of metabolites: the indole derivatives originating from glucobrassicin and the various isothiocyanates including phenethylisothiocyanate from gluconasturtin, and sulforaphanes derived from glucorafanin. The indoles are unique in that they have already been used therapeutically and

will be the focus of this review. Most attention has been given to indole-3-carbinol (I3C) as an estrogen-modulating cancer chemopreventive, despite *in vivo* evidence of ovarian toxicity (2) and tumor promotion (3, 4). Less attention has been directed to 3,3'-diindolylmethane (DIM), a stable product, which is formed from I3C both *in vitro* and *in vivo*.

While the idea of obtaining these compounds from the consumption of cruciferous vegetables (*e.g.* broccoli, cabbage, cauliflower and mustard greens) seemed attractive, the concentration of these compounds present in plants is actually highly variable depending on factors such as the seed strain, myrosinase enzyme activity, rainfall, soil and amount of sunlight. As a result, vegetable or seed extracts are not a practical way to get the daily amounts of these compounds required to be effective. Consequently, most investigators have turned to semisynthetic sources of cruciferous indole compounds, particularly I3C and DIM.

Role of Instability in the Action of Indole-3-carbinol (I3C)

The initial compound released from cruciferous glucobrassicin by the action of the enzyme myrosinase is I3C, a compound that has proved to be highly unstable both *in vitro* and *in vivo* (5). As a result, one cannot be certain whether I3C itself or one of the condensation products arising from I3C is the active agent.

Nevertheless, because of its initial greater availability, most early studies were carried out with I3C without any concern for its stability either as an additive in cell culture studies or orally in animal and human studies. In animal studies, it was shown to have activity against breast tumor formation (6, 7), against endometrial tumors (8), and most recently against prostate cancer growth (9). In cell culture studies and in human studies, I3C was shown to increase 2-hydroxylation of estradiol and decrease 16 α -hydroxylation (10, 11). This change correlated with its antitumor activity. Subsequently, investigators using isolated DIM showed that this single

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conversion product from I3C had similar *in vitro* activity to I3C, *in vivo* activity against transplanted breast (12) and prostate cancer (13, 14), and increased 2-hydroxylation of estradiol in breast cancer survivors (15).

I3C given orally is almost immediately converted to a number of condensation products in the stomach in varying proportions depending upon the exact pH (5). These include a dimer (DIM), trimers including the linear trimer (LTR) 2,3-bis-[3-indolylmethyl]indole; and the cyclic trimer (CTR) (5,6,11,12,17,18-hexahydrocyclonal [1,2-b: 4,5-b':7,8-b:]triindole), the closed ring dimer indolocarbazole (ICZ) [indolo[3,2-b]carbazole], and ascorbigen, the condensation product of I3C and ascorbic acid (5,16). Although DIM predominates, variable amounts of the other products are also formed depending on the exact pH in the stomach or in culture media. At least one of the condensation products (CTR) has shown growth promoting proestrogenic responses and would not be protective (17).

In vitro experiments with rapid addition of I3C to a stirred buffer adjusted to stomach pH resulted in almost instant conversion to the multimers with almost no I3C remaining after a few minutes (unpublished observations from this laboratory). Even in neutral conditions (pH 7.0-7.4) in tissue culture media, I3C is rapidly converted to DIM (18). In addition, attempts to measure plasma I3C after oral administration of I3C to mice showed only a transient blood level of I3C at 15 minutes, when 250 mg/kg was given by gavage (19). When equated to human dosing by allometric scaling, this high dose use corresponds to 20 mg/kg, about 4 times greater than the maximal well tolerated human dose of 5 mg/kg (20). Using a dose of 20 mg/kg of I3C, the proportional plasma peak of I3C based on the observed I3C plasma level using 250 mg/kg in mice would be 0.32 μ M, over 100 times less than the typical concentration of 50 μ M needed to see biological responses from I3C in *in vitro* studies. In human testing, no I3C was found in the plasma even at the earliest 1 hour time point tested after giving single one-time doses up to 17 mg/kg of oral I3C to human volunteers (21). Although in an earlier animal study labeled material was found in the liver following the administration of radioactive I3C, there was no evidence that the intracellular material was I3C rather than its metabolites (22). However, when DIM and LTR levels were measured in blood and tissue in these studies, significant and persistent levels were found (19, 22). The fact that significant histological improvement was seen in 50% of humans with cervical dysplasia treated with oral doses of only 3 and 6 mg/kg of I3C, suggests that one or more of the condensation products are highly active (23). All of these results raise questions about whether the true role of I3C is to serve as a prodrug rather than as the actual therapeutic agent.

Could I3C Act Directly Under Any Circumstances?

Since cancer chemopreventive activity requires exposure over days, weeks and months and there is little to no evidence that

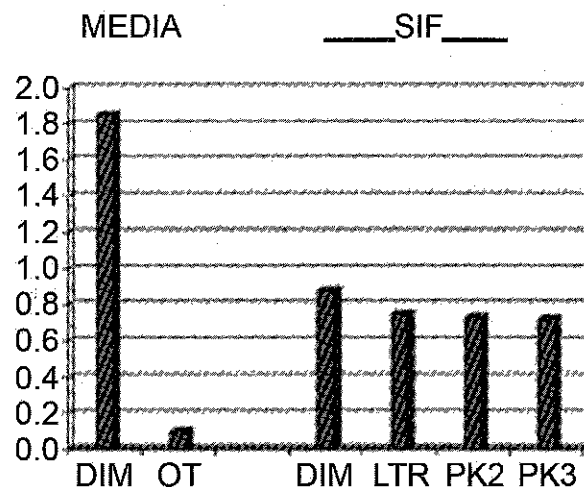


Figure 1. Presence of conversion products relative to the parent indole-3-carbinol in culture media (Media) or synthetic intraperitoneal fluid (SIF) after 24 hours of incubation. DIM, 3,3'-diindolylmethane; LTR, (2,3-bis-[3-indolylmethyl]indole); PK2 peak 2; PK3 peak 3; OT other minor peaks.

I3C survives in cells or in the whole body long enough to modulate cytochrome enzyme activity, steroid receptor activity, or cell cycle dynamics, a direct role for I3C seems implausible. Earlier studies on the intraperitoneal (*i.p.*) administration of I3C showed no response, although *i.p.* injection of the acid reaction products did promote 2-hydroxylation of estrogen (15, 16, 24). The only studies which showed a positive response to an *i.p.* injection of I3C have been reports of the activity of *i.p.* I3C where a significant response in models of prostate cancer was observed (9, 25). To examine the basis for this response, studies incubating I3C in culture media and synthetic intraperitoneal fluid (SIF) and synthetic cerebrospinal fluid were carried out. These have shown rapid, substantial conversion of I3C to only DIM in culture media and to DIM, LTR, and other products in SIF. Conversion of I3C to the trimer was inhibited when the SIF was diluted 1:1 with tissue culture media (18). These findings suggest that the reported results of previous studies using I3C in culture media and giving I3C by *i.p.* injection are probably not due to I3C itself (see Figure 1).

Cell Culture and Animal Studies

In all of the cell culture studies carried out with I3C in the past it was tacitly assumed that I3C was stable in cell culture media over the course of the study period because of the neutral pH. However studies of the stability of I3C in a variety of cell culture media at 37°C showed that there was substantial conversion to DIM by 24 hours (Figure 1) and that the great bulk of I3C had been converted to DIM by 48 hours (18). Furthermore, starting with I3C, only DIM and no I3C were

Table I. Comparison of 2-hydroxyestrogen and 16 α -hydroxyestrone levels before and after absorption-enhanced DIM administration in women with treated breast cancer (47).

| Case# | 2-OHE <DIM | 2-OHE >DIM | 16-OHE1 <DIM | 16-OHE1 >DIM | % Change in 16-OHE1 | 2/16 Ratio <DIM | 2/16 Ratio >DIM | % Change in ratio |
|------------------|---------------|---------------|-----------------|-----------------|------------------------|--------------------|--------------------|----------------------|
| #1 | 22.7 | 19.7 | 23.0 | 8.8 | -61.7 | 0.99 | 2.24 | +126.3 |
| #2 | 38.2 | 29.0 | 20.2 | 6.6 | -67.3 | 1.90 | 4.40 | +131.5 |
| #3 | 10.5 | 21.0 | 20.5 | 12.5 | -39.0 | 0.51 | 1.68 | +229.2 |
| % Change in Meqn | | | | | -56 | | | +162.3 |

ELISA results for urinary 2-hydroxyestrogen (2-OHE) and 16 α -hydroxyestrone (16-OHE1) levels (ng/mg creatinine) and 2-hydroxyestrogen to 16 α -hydroxyestrone ratio (2/16 ratio) in breast cancer cases before (<) and after (>) absorption-enhanced DIM. The ELISA measures both 2-OHE1 and 2-OHE2 equivalently.

found in the nucleus of cultured breast cancer cells (26). This makes it difficult to relate any of the enzyme-inducing effects, DNA repair effects, cell cycle arrest activity, and apoptosis promotion suggested for I3C to be due to I3C itself. Since DIM is at least twice as potent as I3C in cell culture (29), one cannot determine the extent of any direct effects of I3C in tissue culture since there is always enough DIM present in all of the cell culture experiments starting with I3C to account for the biological responses observed. In cell culture conditions, there is minimal to no conversion of I3C to LTR, with DIM as the dominant conversion product (18).

Head to head comparisons of I3C versus DIM by Parkin and Majeska-Griganti (27), Garikapati *et al.* (28), Chen *et al.* (29), Wang *et al.* (30), and others have all shown that DIM is more active than I3C in terms of potency, time to observed effect, ability to suppress CYP450 1B1 and reduce formation of 4-hydroxy estrogens. Following chronic treatment of Sprague-Dawley rats with either I3C or absorption-enhanced DIM, no toxic responses were observed in rats given DIM, while reversible hepatocyte hypertrophy and P-450 enzyme induction was observed in those rats given I3C (31). The effects lasted after the treatment was stopped and only slowly regressed. In a one-year chronic feeding study in rats, Leibelt *et al.* also showed that DIM was safer causing less liver enlargement than I3C (32). Various investigators found that oral administration of I3C or ascorbigen resulted in increased production of 4-hydroxyestrogen metabolites (3, 33-35). The increase in 4-hydroxyestrogen production in humans given I3C is of great concern regarding safety, even though the increase did not reach statistical significance in pilot studies (35). The presence of increased levels of CYP1B1, which produces 4-hydroxyestrogen, has already been shown in human breast cancer tissue by Singh *et al.* (36). This is even more important in women who smoke, where elevated production of 4-hydroxyestrogen (37) and increased breast cancer occurrence (38) has already been documented. Recently, the combination of I3C and tobacco smoke in animals was shown to lead to fetal toxicity (39). Increased production of

4-hydroxyestrogen is a risk factor for both breast and prostate cancer, as has been shown by Cavalieri *et al.* (40).

The one clear case of I3C being active where DIM was not is in protection against the hepatotoxic effects of trabectedin, an anti-breast cancer drug (41). I3C clearly worked here but DIM did not. One must assume that one of the other multimers (LTR, CTR, ICZ, and/or ascorbigen) formed in the stomach from I3C is responsible for the effect in this animal study. Since the protection here is due to the induction of CYP3A4 enzymes, which enhance the clearance of the drug, this is not necessarily a beneficial activity for I3C. That I3C results in induction of 3A4, while DIM does not, clearly makes I3C undesirable for long-term treatment in which its use could affect the blood levels of oral contraceptives and other drugs being taken concurrently, a result that the FDA and other drug regulating agencies consider unacceptable.

The argument proposed by Rogan (42) that I3C will better lower 16-hydroxyestrogen production than DIM ignores I3C-induced concurrent increased formation of 4-hydroxyestrone, a known carcinogen (40). The increased side-effects from any increase in CYP3A4 more than counterbalance any proposed benefits of I3C over DIM. Side by side animal studies of I3C and absorption-enhanced DIM showed that I3C induced CYP3A4 and DIM did not (43). In a preliminary evaluation in women with breast cancer and elevated urinary 16-hydroxyestrone, Zeligs *et al.* have reported significant reduction in urinary 16-hydroxyestrone levels using only absorption-enhanced DIM (44) (Table I).

Concluding Perspective

In light of this cumulative and recent evidence on the conversion of I3C to DIM in cell culture, peritoneal fluid, and with oral use, and in view of substantial direct activity seen with DIM, there is no longer a case for considering I3C to be directly active. In view of the evidence that non-DIM digestive products from I3C are associated with greater enzyme induction than DIM, questions of safety surround the chronic

use of I3C as a dietary supplement. The fact that one month human use of 400 mg/day of I3C showed induction of CYP1A2 (45) does not indicate safety for chronic I3C use, since CYP1A2 is now known to be a source of 4-hydroxyestrogen (46). The documented increase in carcinogenic 4-hydroxyestrogen following oral use of I3C in animals (34) and humans (11) should discourage the use of I3C as an acceptable chemopreventive supplement. Based on evidence of bioavailability (19), clinical studies (15, 47), and studies showing reduced 4-hydroxyestrogen production with absorption-enhanced DIM (27), DIM supplementation is a promising alternative to the use of I3C. One must keep in mind that because of its greater initial availability, all of the initial studies in our and other laboratories were carried out with I3C. These promising studies lead to further work showing the advantages of using DIM, a dimer of I3C, as the drug of choice.

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