Science Review: Indole-3-carbinol (I3C) and 3,3'-diindolylmethane (DIM)

Introduction

Large-scale epidemiological analyses have found an inverse association between intake of cruciferous vegetables (e.g., broccoli, cabbage, cauliflower and Brussels sprouts) and risk of cardiovascular disease, some cancers, and all-cause mortality.[1,2] Cruciferous vegetables are a rich source of a variety of bioactive compounds, including sulfur-containing phytochemicals known as glucosinolates.[3] Glucosinolates may have evolved as a form of defense; when a glucosinolate-containing plant is mechanically damaged (e.g., by chewing), the molecule is exposed to and subsequently hydrolyzed by the enzyme myrosinase. The resulting breakdown products include isothiocyanates, thiocyanates, and epithionitriles, many of which have been evaluated for their bioactivity.[4] One particularly interesting hydrolyzed product is indole-3-carbinol (I3C).[3] I3C has been found to have numerous biological activities, including effects on cell division, angiogenesis, and hormone metabolism.[5]

I3C is an unstable compound in acidic conditions and undergoes rapid oligomerization to form a mixture of acid condensation products, with 3,3'-diindolylmethane (DIM) being the predominant compound.[6] However, human feeding studies reveal that, after subjects ingest I3C, DIM is the only detectable I3C-derived compound in plasma.[7] Scientists believe that many of the biological effects of I3C are attributable to DIM.

As a dietary ingredient, DIM is poorly absorbed from the gastrointestinal tract due to its lipophilic property.[8] However, certain technologies have been applied to DIM to help improve its bioavailability.[9] The absorption-enhanced DIM has been evaluated for its effects in several human studies.[10-13]

Research Highlights

- Cruciferous vegetables are a rich source of glucosinolates, which are broken down into bioactive metabolites by the plant enzyme myrosinase. One of the metabolites with therapeutic interest is I3C.[3]
- Human feeding studies demonstrate that I3C is rapidly converted to DIM, the only detectable I3C-derived compound in plasma.[7]
- Both I3C and DIM exert various biological activities, such as:[2,14-18]
  - Modulating phase I and phase II biotransformation such as increased CYP1A2 activity
  - Modulating estrogen metabolism toward production of 2-hydroxyestrone (2OHE1) at the expense of 16α-hydroxyestrone (16αOHE1), resulting in a higher ratio of 2OHE1 to 16αOHE1
  - Affecting other signaling pathways that may contribute to chemoprevention

Mechanisms of Action

Several molecular mechanisms of I3C and/or DIM have been proposed:

Modulation of biotransformation

Biotransformation enzymes play an important role in the metabolism and elimination of not only numerous endogenous compounds but also exogenous compounds including environmental carcinogens and toxins.[19] I3C and DIM have been demonstrated to bind to the transcription factor aryl hydrocarbon receptor (AhR), allowing AhR to enter the nucleus to form a complex with xenobiotic response elements (XREs) of target genes. This leads to upregulation of genes that contain XREs, including genes of phase I (CYP) and phase II biotransformation enzymes.[14] I3C and DIM have also been shown to bind to the nuclear factor erythroid 2-related factor 2 (Nrf2), resulting in activation of the Nrf2 signaling pathway and upregulation of genes for phase II biotransformation enzymes and antioxidant enzymes.[15]

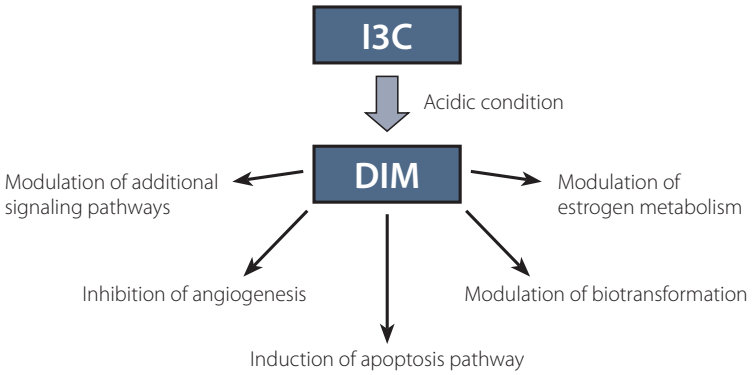
Modulation of estrogen metabolism

Via cytochrome P450-dependent pathways, endogenous estrogens such as 17β-estradiol (E2) and estrone (E1) are irreversibly converted to two major metabolites: 2OHE1, which is believed to have anti-estrogenic effects,[20] and 16αOHE1, which has been shown to induce abnormal cell proliferation.[21] I3C and DIM upregulate genes for CYP enzymes that result in increases in 2OHE1 at the expense of 16αOHE1.[16] It has been proposed that a higher ratio of 2OHE1 to 16αOHE1 (2:16 ratio) indicates a shift in metabolic pathway toward 2OHE1 from the 16αOHE1 and may be associated with reduced risks of estrogen-sensitive cancers.[22]

Effects on signaling pathways

I3C and DIM have also been demonstrated *in vitro* to target several other signaling pathways that are involved in cancer pathophysiology. For example:

- In several cancer cell lines, pretreatment with DIM has been shown to inhibit NF-κB activity, leading to inhibition of cell growth and induction of programmed cell death.[2,18]
- I3C and particularly DIM inhibit angiogenesis via inactivation of ERK1/2 and/or Akt signaling in endothelial cells.[17]



Human pharmacokinetic studies of I3C and DIM

I3C

- In female subjects, single-dose oral administration of I3C (400-1200 mg) yielded DIM as the circulating product, and no I3C was detected in plasma.[7]
- Peak plasma concentration of DIM following ingestion of 400 mg I3C was 61 ng/mL. Peak concentration occurred at approximately 2 hours and was no longer detectable after 24 hours.[7]
- Multidose study indicated that neither extensive bioaccumulation nor enhanced clearance due to enzyme induction occurred with I3C.[7]

DIM

- In healthy subjects, single-dose oral administration of absorption-enhanced DIM (50-300 mg) demonstrated a linear increase of DIM in plasma. The single 200 mg produced a peak plasma DIM concentration of 104 ng/mL and was no longer detectable after 24 hours.[23]
- Multidose pharmacokinetic studies of absorption-enhanced DIM are lacking. One small preliminary study noticed a drop in plasma DIM concentration after 4 weeks of consumption, although the investigators acknowledged that the plasma DIM level might not reflect the tissue level.[12]
- In a study in which women received absorption-enhanced DIM (108 mg daily for 30 days), urinary levels of DIM were significantly increased indicating DIM could be excreted via urine.[10] However, the major route of DIM excretion in humans remains to be investigated.[24]

	I3C	DIM
Source	Formed as a hydrolysis product of glucobrassacin (a glucosinolate compound found in cruciferous vegetables)	Formed from I3C as an acid condensation product in an acidic environment (e.g., stomach)
Dose exerting efficacy based on clinical trials	300-400 mg per day[25-30]	(Absorption-enhanced DIM) 100-300 mg per day[10,11,13]
Activity	Converts into DIM which exerts biological activity	Exerts biological activity
Clinical health benefits	Modulation of biotransformation and estrogen metabolism (e.g., a shift in metabolic pathway toward 2OHE1 from the 16αOHE1) and other potentially chemopreventive activities	
Safety	• Well tolerated at up to 400 mg twice daily for 8 weeks[7] • Long-term effects of I3C on cancer risk in humans are not known	• (Absorption-enhanced DIM) Well tolerated at 300 mg daily for 12 months in women taking tamoxifen, with some discolored urine reported[13] • Long-term effects of absorption-enhanced DIM on cancer risk in humans are not known

Clinical evidence of I3C and DIM

I3C

- Several human clinical studies have demonstrated that oral supplementation with I3C (300-400 mg per day, ranging from 4 weeks to 3 months) significantly increases urinary 2OHE1 levels and/or urinary 2:16 ratio in various (predominantly female) populations.[25-30]
- In a 4-week study, the maximal increase in CYP1A2 enzyme activity was seen with the 400 mg daily dose of I3C.[30]
- In a prospective open-label study in patients with respiratory papillomatosis taking 200 mg of I3C daily, 63% of participants either had remission of papillomatous growth that resulted in no surgery or less frequent surgery.[31]

DIM

- In a pilot study with postmenopausal women with a history of early-stage breast cancer, oral supplementation with absorption-enhanced DIM (108 mg per day for 30 days) resulted in significant increases in urinary levels of 2OHE1 and cortisol and a borderline increase in 2:16 ratio.[10]
- In a pilot study with subjects with thyroid proliferative disease, oral supplementation with absorption-enhanced DIM (300 mg per day for 14 days) helped increase DIM concentration in thyroid tissues and enhanced estrogen metabolism as indicated by increased 2:16 ratio.[11]
- In a randomized controlled trial involving women taking tamoxifen for breast cancer, daily supplementation of absorption-enhanced DIM (140 mg twice daily for 12 months) significantly increased urinary 2:16 ratio and serum levels of sex hormone-binding globulin (SHBG) compared with placebo.[13] Epidemiological evidence suggests that higher SHBG may be a protective factor of breast cancer in postmenopausal women.[32]

References:

1. Aune D et al. *Int J Epidemiol*. 2017;46(3):1029-1056.
2. Higdon JV et al. *Pharmacol Res*. 2007;55(3):224-236.
3. Holst B et al. *Nat Prod Rep*. 2004;21(3):425-447.
4. Ishida M et al. *Breed Sci*. 2014;64(1):48-59.
5. Licznerska B et al. *Adv Exp Med Biol*. 2016;928:131-154.
6. Maciejewska D et al. *Eur J Med Chem*. 2009;44(10):4136-4147.
7. Reed GA et al. *Cancer Epidemiol Biomarkers Prev*. 2006;15(12):2477-2481.
8. Anderton MJ et al. *Drug Metab Dispos*. 2004;32(6):632-638.
9. Leibelt DA et al. *Toxicol Sci*. 2003;74(1):10-21.
10. Dalessandri KM et al. *Nutr Cancer*. 2004;50(2):161-167.
11. Rajoria S et al. *Thyroid*. 2011;21(3):299-304.
12. Reed GA et al. *Cancer Research*. 2010;70(8 Suppl):2899-2899.
13. Thomson CA et al. *Breast Cancer Res Treat*. 2017;165(1):97-107.
14. Safe S. *Toxicol Lett*. 2001;120(1-3):1-7.
15. Saw CL et al. *Biopharm Drug Dispos*. 2011;32(5):289-300.
16. Michnovicz JJ et al. *J Natl Cancer Inst*. 1990;82(11):947-949.
17. Kunimasa K et al. *J Nutr*. 2010;140(1):1-6.
18. Maruthanila VL et al. *Adv Pharmacol Sci*. 2014;2014:832161.
19. Sudhakaran S et al. In: *Bioresources and Bioprocess in Biotechnology: Vol 2 : Exploring Potential Biomolecules*. Springer Singapore;2017:129-150.
20. Schneider J et al. *J Biol Chem*. 1984;259(8):4840-4845.
21. Fishman J et al. *J Clin Endocrinol Metab*. 1980;51(3):611-615.
22. Bradlow HL et al. *Environ Health Perspect*. 1995;103 Suppl 7:147-150.
23. Reed GA et al. *Cancer Epidemiol Biomarkers Prev*. 2008;17(10):2619-2624.
24. Fujioka N et al. *Cancer Epidemiol Biomarkers Prev*. 2014;23(2):282-287.
25. Bradlow HL et al. *Cancer Epidemiol Biomarkers Prev*. 1994;3(7):591-595.
26. McAlindon TE et al. *Lupus*. 2001;10(11):779-783.
27. Michnovicz JJ et al. *J Natl Cancer Inst*. 1997;89(10):718-723.
28. Michnovicz JJ. *Int J Obes Relat Metab Disord*. 1998;22(3):227-229.
29. Wong GY et al. *J Cell Biochem Suppl*. 1997;28-29:111-116.
30. Reed GA et al. *Cancer Epidemiol Biomarkers Prev*. 2005;14(8):1953-1960.
31. Rosen CA et al. *J Voice*. 2004;18(2):248-253.
32. He XY et al. *Horm Metab Res*. 2015;47(7):485-490.