Single-Dose Pharmacokinetics and Tolerability of Absorption-Enhanced 3,3′-Diindolylmethane in Healthy Subjects

Gregory A. Reed,[1,2,5] Jean M. Sunega,[2] Debra K. Sullivan,[3,5] John C. Gray,[5] Matthew S. Mayo,[4,5] James A. Crowell,[6] and Aryeh Hurwitz[1,2,5]

Departments of [1]Pharmacology, Toxicology, and Therapeutics; [2]Internal Medicine; [3]Dietetics and Nutrition; and [4]Biostatistics, and the [5]Kansas Masonic Cancer Research Institute, University of Kansas Medical Center, Kansas City, Kansas; and [6]Chemopreventive Agent Development Research Group, National Cancer Institute, Bethesda, Maryland

Abstract

We have completed a single ascending dose clinical study of the proposed chemopreventive agent 3,3′-diindolylmethane (DIM). The study agent was nutritional-grade, absorption-enhanced BioResponse 3,3′-diindolylmethane (BR-DIM). We determined the safety, tolerability, and pharmacokinetics of single doses of BR-DIM in drug-free, non-smoking, healthy men and women. Groups of four subjects were enrolled for each dose level. After randomization, one subject in each group received placebo whereas three received active BR-DIM. The doses administered were 50, 100, 150, 200, and 300 mg, with the 300-mg dose repeated in an additional group. No BR-DIM–related adverse effects were reported at doses up to 200 mg. At the 300-mg dose, one of six subjects reported mild nausea and headache and one also reported vomiting. Only the latter effect was judged as probably related to the study agent. Analysis of serial plasma samples showed that only one subject at the 50-mg dose had detectable concentrations of DIM. The single 100-mg dose of BR-DIM resulted in a mean maximum plasma concentration (C_{max}) of 32 ng/mL and a mean area under the curve (AUC) of 128 h ng/mL, and a single 200-mg dose produced a mean C_{max} of 104 ng/mL and a mean AUC of 553 h ng/mL. The single 300-mg dose of BR-DIM resulted in a mean C_{max} of 108 ng/mL and a mean AUC of 532 h ng/mL. We conclude that BR-DIM is well tolerated at single doses of up to 200 mg, and that increasing the dose to 300 mg did not result in an increase in C_{max}. (Cancer Epidemiol Biomarkers Prev 2008;17(10):2619–24)

Introduction

Cruciferous vegetables such as cabbage, broccoli, cauliflower, and brussel sprouts contain several chemicals that have been shown to modulate carcinogenesis in animals (1-10) and humans (11, 12). Among these compounds is glucobrassicin (3-indolylmethyl glucosinolate). Glucobrassicin undergoes autolysis, forming indole-3-carbinol (I3C), indole-3-acetonitrile, and 3,3′-diindolylmethane (DIM), a dimer of I3C. I3C has been shown to have pronounced chemopreventive effects against the development of both spontaneous (4, 6) and chemically-induced (1-3, 5, 7-10) tumors in rats, mice, and trout. These chemopreventive effects of I3C were reported against tumor development in the mammary gland (1, 4, 7), liver (2, 3), lung (5, 8), cervix (6, 9), and gastrointestinal tract (1). Our previous study of the tolerability, pharmacokinetics, and effects of oral I3C in humans showed that the only detectable circulating product was DIM (13).

Our observations that no I3C was detectable in the plasma of women ingesting the compound, that DIM was the only I3C-derived compound detected (13), and that the ingestion of I3C elicited changes in drug and estrogen metabolism consistent with proposed chemoprevention mechanisms (14), when coupled with other reports supporting DIM as a major active chemopreventive agent derived from I3C (15-17), led to this study of DIM itself as a possible chemopreventive supplement. DIM, however, is poorly absorbed from the gastrointestinal tract. To address this potential problem, a formulation of DIM was administered that provides increased bioavailability of the compound, as shown by preliminary preclinical (18, 19) and clinical studies (17). We are studying Nutritional-grade BioResponse-DIM (BR-DIM), the absorption-enhanced DIM formulation, in healthy men and women in a Phase I clinical trial. As reported here, we examined the safety and tolerability of single ascending doses of BR-DIM in drug-free men and women found healthy by medical histories, physical examinations, and a battery of blood and urine tests. We also determined the pharmacokinetics of BR-DIM in these subjects. Based on the safety and tolerability of single doses of BR-DIM determined in this study, a separate protocol has been developed for a multiple-dose study of BR-DIM. In addition to studying the safety, tolerability, and pharmacokinetics of single and multiple doses of BR-DIM, the follow-up protocol will evaluate the effects of BR-DIM on enzymes controlling the disposition of clinically used drugs, toxicants, and steroids.

Received 6/5/08; revised 7/22/08; accepted 7/30/08.

Grant support: National Cancer Institute contract N01-CN-35008.

Note: A. Hurwitz, deceased.

Requests for reprints: Gregory A. Reed, MS 1018, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. Phone: 913-588-7513; Fax: 913-588-7501. E-mail: greed@kumc.edu

doi:10.1158/1055-9965.EPI-08-0520

Materials and Methods

Test Compounds. DIM, BR-DIM (50 mg DIM per capsule; BioResponse, LLC), and matching placebo capsules were supplied by the Division of Cancer Prevention, National Cancer Institute. BR-DIM is a patented oral formulation containing D-α-tocopheryl acid succinate, phosphatidylcholine, and silica microencapsulated in starch (20). This formulation exhibits higher bioavailability than does crystalline DIM (19).

Subjects and Treatments. Men and women ages 22 to 58 y who had negative results for tobacco use, based on urine cotinine, and for a drug screen, were enrolled. All enrolled subjects were free of acute, unstable, chronic or recurring medical conditions and with calculated body mass index between 18 and 30. The characteristics of the enrolled subjects are provided in Table 1. Strict vegetarians or individuals who ate more than 3 medium servings (one half cup each) of cruciferous vegetable per wk were excluded. Those who stopped ingesting cruciferous vegetables ≥14 d and alcohol ≥7 d before starting this study were not excluded. Participants completed a brief diet questionnaire to assess these criteria. Caffeine- and grapefruit-containing foods and beverages were avoided by subjects for at least 48 h before BR-DIM dosing. All subjects had blood and urine chemistries and complete blood cell counts within the reference ranges, and all women had negative pregnancy tests before BR-DIM administration. All protocols, procedures, informed consent, and other forms were reviewed and approved by the Human Subjects Committee of the University of Kansas Medical Center.

Dosing and Sampling Procedures. The subjects, who had fasted overnight, had an intravenous catheter inserted and then were administered their oral dose of BR-DIM or matching placebo with 200 mL water. Fasting was continued for an additional 2 h to allow for the absorption of DIM. Blood samples were collected into heparinized tubes immediately before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing for determination of plasma DIM concentrations. Samples were immediately placed in ice, and plasma was prepared within 30 min of sampling. Plasma samples were stored below −70°C, and analyzed within 4 mo of acquisition. Subjects were observed and vital signs monitored during the first 12 h and at the 24-h sampling time.

Plasma DIM Analysis. All samples were analyzed under contract at the School of Pharmacy, Texas Tech University-Health Sciences Center, Dallas, Texas. The plasma samples were analyzed for DIM by liquid chromatography-mass spectrometry using a validated method (13), which involves the extraction of DIM from human plasma into diethyl ether. Indole-3-ethanol (I3E) was used as the internal standard. Separation was achieved by reverse phase high performance liquid chromatography. The mass spectrometer was operated in the selected ion recording mode with ionization via positive ion electrospray. Ions with a m/z value of 130 Da (DIM), 162.1 Da (I3E parent ion, M+1), 247 Da (DIM parent ion, M+1), and 263 Da [hydroxylated DIM parent ion, (M+1)] were monitored.

Pharmacokinetic Analysis. The data were analyzed using non-compartmental methods (WinNonlin, Version 5.1, SCI). Plasma concentration data were analyzed separately for each subject using this model-independent method of pharmacokinetic analysis to obtain area under the concentration-time curve up to the last measurable time point (AUC_{0-t}), maximum plasma concentration (C_{max}), and time to reach C_{max}. Additionally, plasma concentrations for each individual were analyzed to provide an estimate of the elimination half-life for the DIM. AUC_{0-t} was calculated using the trapezoidal rule.

Table 1. Subject characteristics

Subject	Age	Sex	Weight, pounds	Height, inches	Body mass index	Study drug dose, mg
201	38	Female	157	62	28.8	50
202	55	Female	172	66	27.8	50
203	24	Female	159	68	24.2	50 (P)
204	23	Male	166.5	74	21.4	50
206	23	Male	149	71	20.8	100
207	58	Female	144.5	65	24.1	100
208	23	Male	154.4	71	21.6	100 (P)
209	25	Female	169	69	25.0	150
210	22	Male	139.4	72	19.0	100
211	25	Male	211.5	74	27.2	150 (P)
212	25	Male	204	73	27.0	150
213	25	Male	128	70	18.4	150
215	24	Female	125	62	22.9	200
216	54	Female	131	64	22.5	200 (P)
217	47	Female	111	64	19.1	200
218	25	Male	182.5	72	24.8	300
219	25	Male	225.5	78	26.1	200
220	30	Female	138.5	66	22.4	300
222	28	Male	184.5	73	24.4	300
223	24	Female	146	64	25.1	300 (P)
225	28	Male	161	71	22.5	300 (P)
226	44	Male	191	67	30.0	300
227	44	Male	167	71	23.3	300
228	44	Female	136	67	21.3	300

Abbreviation: P, placebo.

Table 2. Cumulative listing of adverse events

Subject	Study drug dose, mg	Event	Event grade	Event duration, d	Relatedness/attribution
210	100	Headache	1	1	Unlikely
209	150	Flatulence, menstrual spotting	1	2	Unlikely
216	Placebo	Blurred vision, headache, dizziness	1	1	Possibly
		Loss of appetite, nausea			
		Vomiting			
219	200	Flatulence	1	3	Possibly
222	300	Headache, nausea	1	1	Possibly
228	300	Vomiting	1	1	Probably
		Nausea		2	

Results

We completed dosing of subjects with single ascending doses of BR-DIM. There were 6 groups of 4 subjects each (3 DIM and 1 matching placebo in each group). The treatment groups received 50, 100, 150, 200, and 300 mg DIM, with a second group receiving the highest (300 mg) dose. All adverse events in these subjects are listed in Table 2. One subject reported headache and nausea and another became nauseated and vomited after receiving a 300-mg BR-DIM dose. In addition, one subject complained of nausea and headache, but was found after breaking the randomization code to have received placebo, rather than active BR-DIM. Two subjects, one receiving a 150-mg dose and the other a 200-mg dose of BR-DIM, complained of flatulence. All adverse events were classified as grade I, which is defined as mild, self-limiting, and not requiring treatment. Based on subject examination and on consideration of the time of onset and resolution of adverse effects relative to DIM administration, only the vomiting after the 300-mg dose was classified as probably related to DIM.

Serial plasma samples from all subjects were analyzed by liquid chromatography-mass spectrometry for DIM concentration, and pharmacokinetic variables were calculated. Mean plasma DIM concentrations over sampling time are shown for each dose group in Fig. 1. No subjects had detectable DIM in their pre-dose plasma, and all subjects' DIM plasma concentrations had dropped to below our limit of detection by the 12-h time point for BR-DIM doses of 150 mg or less, and at 24 h for all doses.

The pharmacokinetic variables C_{max} and time to reach C_{max} were determined for each subject by inspection, and AUC and elimination half-life values were calculated for each individual subject. Mean values are reported for all doses except the 50-mg dose, where only one subject showed detectable DIM concentrations in plasma. These values are presented in Table 3, and their dose-dependence is presented in Fig. 2. The time to reach C_{max} and the elimination half-life values for DIM vary somewhat but show no trend as a function of BR-DIM dose. C_{max}, however, increases in a linear fashion at least up to the 200-mg dose (Fig. 2A), and AUC is a linear function of dose throughout the dose range tested (Fig. 2B). These findings may be compared with our results for DIM in plasma obtained when the DIM precursor I3C was administered orally to women (13), where both C_{max} (Fig. 2C) and AUC (Fig. 2D) increased as a linear function of dose only at the lowest two doses of I3C, and then increased 8 to 12 times faster with increasing dose, compared with the relationship seen at the low doses.

When all plasma samples had been analyzed for DIM concentration and pharmacokinetic variables had been calculated, all subjects were assessed for the relationship between C_{max} and AUC for DIM and the occurrence of adverse effects. As shown in Fig. 3, no clear relationship or trend could be seen between either pharmacokinetic variable and the incidence or severity of reported adverse effects. Even if the reported adverse effects from the subject receiving placebo are excluded, the remaining C_{max} and AUC values appear clustered in the middle of the overall calculated ranges for these variables. Subject 228, who exhibited the only adverse effect classified as probably related to BR-DIM, was found to have a C_{max} of 82 ng/mL and an AUC of 272 h ng/mL. Both of these values are exceeded in several subjects who reported no adverse effects. Whether this lack of correspondence between either AUC or C_{max} for DIM with the occurrence of adverse effects is due to individual variability in

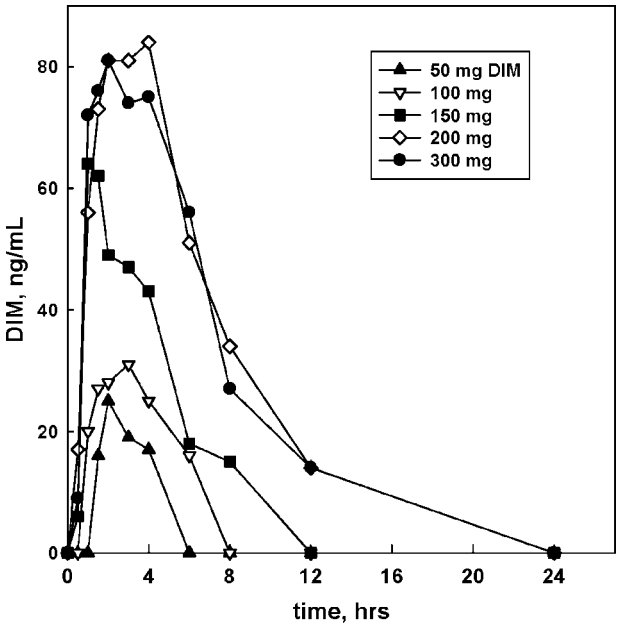

Figure 1. Mean plasma concentration profiles for single doses of BR-DIM. Values are the actual plasma concentrations of DIM for the single subject at the 50-mg BR-DIM dose with measurable plasma concentrations, and the mean values for all dosed subjects for the 100-, 150-, and 200-mg doses ($n = 3$ per dose) and the 300-mg dose ($n = 6$) of BR-DIM.

Table 3. Single-dose pharmacokinetic variables for BR-DIM

N	Dose, mg	t_{max}, h*	$t_{1/2}$, h*	C_{max}, ng/mL*	AUC, h ng/mL*
1†	50	2.0	3.5	25.	62
3	100	2.7 ± 0.6	3.7 ± 1.2	32. ± 4	136 ± 8
3	150	1.8 ± 1.0	3.0 ± 0.6	79. ± 41	314 ± 249
3	200	2.5 ± 1.3	2.6 ± 0.7	104. ± 94	417 ± 441
6	300	2.2 ± 1.1	4.5 ± 1.4	108. ± 43	532 ± 289

Abbreviations: t_{max}, time to C_{max}; $t_{1/2}$, elimination half-life.
*Data are expressed as mean ± SD.
†Only one subject showed detectable DIM in plasma at this dose.

susceptibility, or if it denotes that DIM did not actually cause those effects, cannot be determined from our study.

Discussion

Our results show that single doses of BR-DIM of up to 200 mg are well tolerated by healthy subjects, and that even at a dose of 300 mg adverse effects were infrequent and of minimal severity. Although adverse effects were observed, several features suggest that these effects may not be caused by DIM. First, the time of onset and the duration of some reported adverse effects are difficult to associate with the time of BR-DIM administration and its pharmacokinetics. Moreover, the incidence of adverse effects does not correlate with BR-DIM dose (Table 2). As shown in Fig. 3, the incidence of adverse effects also does not correlate with actual DIM exposure, based either on C_{max} or on AUC. Finally, the most extensive range of adverse effects was from a subject who had received placebo, rather than active BR-DIM.

This tolerability of BR-DIM is consistent with the results of a pilot study in which 19 postmenopausal women took BR-DIM (108 mg DIM daily) for 30 days (17). Of those subjects, one reported a rash that was resolved with discontinuing the supplement and taking antihistamines, two reported increased hot flashes but continued to take the supplement, and one subject complained of nausea when BR-DIM was taken without food. BR-DIM also has been well tolerated in our ongoing multiple-dose study. To date, 14 healthy subjects have received twice-daily doses of either 100 mg or 200 mg BR-DIM for a 4-week period. This is a double-blind study, so no assignment by dose can be made before the completion of all analyses, but adverse effects have not been reported by our subjects. The results of the pilot study in postmenopausal women (17), of our single ascending dose study reported here, and the preliminary findings of our current multiple-dose study with BR-DIM all support the tolerability of BR-DIM in this dose range.

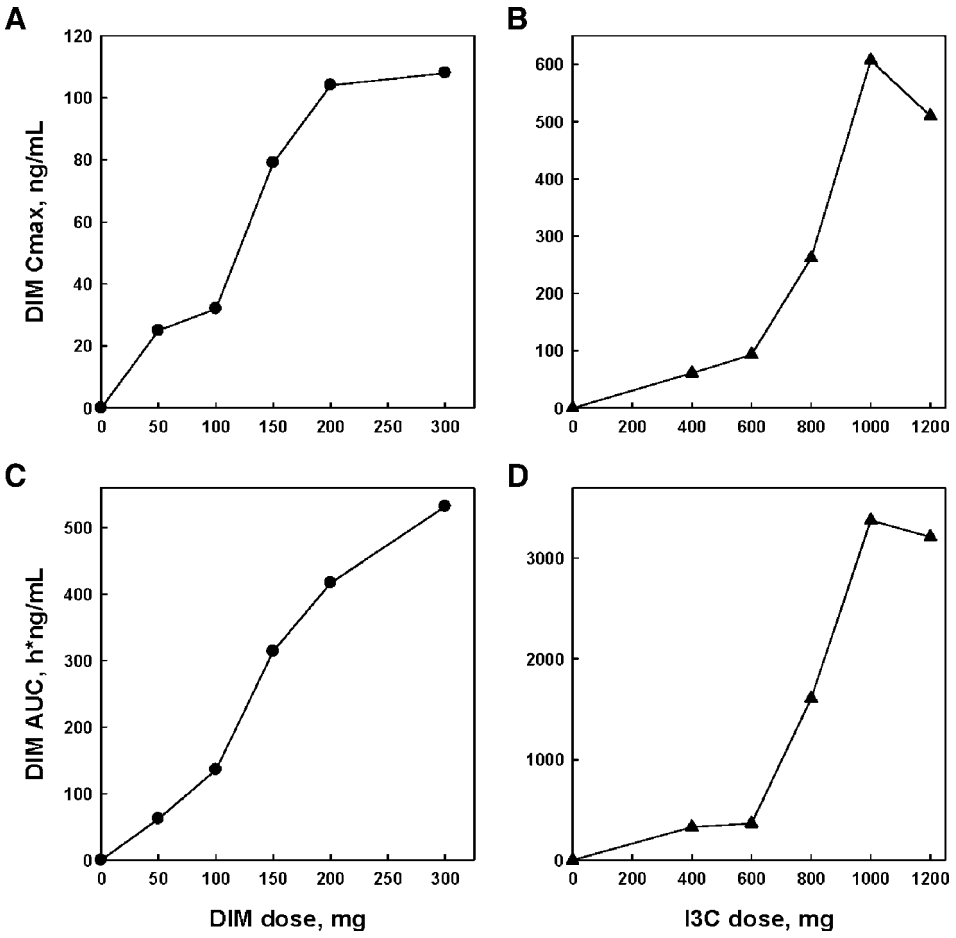

Figure 2. Dose-dependency of DIM pharmacokinetics following BR-DIM and I3C administration. The observed C_{max} and the calculated AUC for DIM from the administration of different doses of BR-DIM (this study; *A* and *C*, respectively) or from different doses of I3C (ref. 13; *B* and *D*, respectively). Values from subjects receiving BR-DIM (*A* and *C*) are from a single subject at the 50-mg dose, and the mean values for three subjects at the 100-, 150-, and 200-mg doses and six subjects at the 300-mg dose. For subjects receiving I3C (*B* and *D*), values shown are the means of four subjects from the 400-, 600-, and 800-mg doses; three subjects at the 1,000-mg dose, and eight subjects at the 1,200-mg dose.

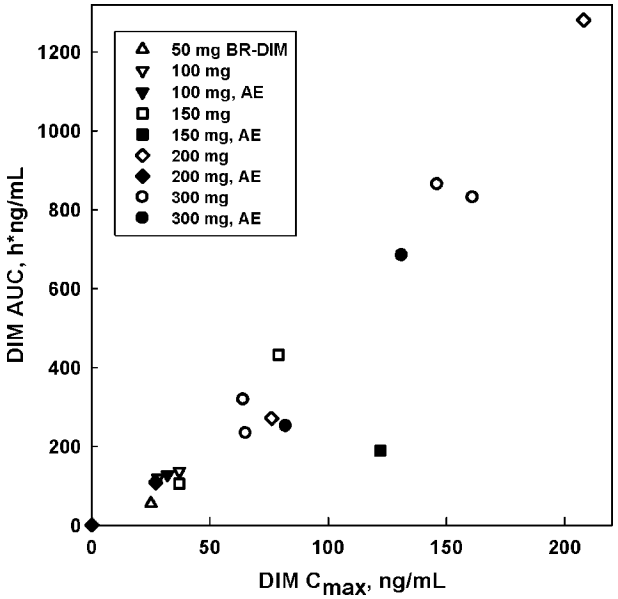

Figure 3. Relationship between DIM plasma concentrations and adverse events. Points represent the C_{max} and AUC for all subjects, with each subject's dose of BR-DIM denoted as labeled. Filled symbols denote the six subjects reporting adverse effects, as listed in Table 2.

Although most studies on the effects of I3C and related compounds on carcinogenesis in animal models have shown chemopreventive effects (1-10), there have been reports of apparent tumor promotion by I3C in certain systems and at relatively high doses (21-23). A single report of tumor promotion by DIM, in an aflatoxin B1–initiated liver tumor model in trout, also has been published (24). A unifying feature of the studies showing tumor promotion by either I3C or DIM is the use of high doses of these supplements, and these doses seem to be associated with toxicity (25). These reports of possible tumor-promoting activity for these indole derivatives is noteworthy, but the high doses used in those studies and the associated toxicity both differ from the dosing regimens we have used and from our observations on tolerability and adverse effects reported here and in our current multiple-dose study.

Examination and calculation of pharmacokinetic variables for DIM from BR-DIM produce a time to reach C_{max} and an elimination half-life similar to those for DIM following ingestion of I3C (13). A comparison of C_{max} and AUC values, however, shows that when normalized to dose administered, BR-DIM produces 2 to 3 times higher values than does I3C. This could represent both the fractional conversion of I3C to DIM under acid conditions (26) and the lower bioavailability of DIM without the absorption-enhancing BR-DIM formulation.

Our inclusion and exclusion criteria were intended to minimize differences in general health, diet, social habits, and drug exposure as variables affecting DIM pharmacokinetics. Despite these restrictions, marked interindividual variability in C_{max} and AUC for DIM were noted (Table 2). When these values were compared among subjects at a given dose level, no correlation or trend could be found with sex, age, or body mass index. Normalizing C_{max} and AUC to dose, expressed as mg/kg body weight, had minimal effects on the coefficient of variation for each dose group. Interindividual variability in DIM metabolism might also contribute to the observed variability in pharmacokinetic variables, but DIM metabolites have not been reported from *in vivo* studies. Staub et al. recently reported the formation of hydroxylated DIM sulfates by human breast cancer cells in culture (27), but the relevance of this finding to the intact organism is not known. It is noteworthy that Anderton et al. did not observe any DIM metabolites in either tissues or plasma of mice dosed with I3C (28) or DIM (19). The relative contributions to interindividual variability in DIM pharmacokinetics of genetic variability and of additional dietary or environmental factors not addressed by our criteria cannot be assessed from this study.

Our pharmacokinetics data show a more linear dose-exposure relationship for BR-DIM, over the range from 50 mg to 300 mg, than was observed using the DIM precursor I3C (13). The mean C_{max} for DIM is a linear function of BR-DIM up to the 200-mg dose ($r^2 = 0.9552$), and the mean AUC is a linear function of BR-DIM dose up to the 300-mg dose ($r^2 = 0.9682$). In contrast, DIM C_{max} and AUC following ingestion of I3C deviated dramatically from linearity (Fig. 2). Such markedly dose-dependent pharmacokinetics presents a major challenge to standardization of dose and predictability of responses, thus the linearity of pharmacokinetics supports BR-DIM as the more favored supplement for development as a chemopreventive agent.

The linearity of BR-DIM pharmacokinetics shown here prompts a reexamination of our consideration of DIM pharmacokinetics when I3C, the precursor, is administered (13). We suggested that the increasing dose-normalized C_{max} and AUC at increasing doses of I3C could represent a saturation of MDR1 and other efflux transporters in the enterocytes, thus increasing the net uptake of DIM. The increased linear range of C_{max} and AUC with BR-DIM administration suggests that saturation of efflux is less likely. Rather, the superlinear increases observed with I3C may reflect increased DIM formation in a bimolecular reaction of I3C-derived reactants to produce DIM.

In our phase 1 study of I3C, we noted that DIM was the only detectable I3C-derived compound in plasma, and that no adverse effects were reported or observed at doses of 200 and 400 mg administered twice daily for four weeks (13, 14). The latter dose generated a C_{max} of 69 ± 42 ng/mL and an AUC of 372 ± 180 h ng/mL (13). These C_{max} and AUC determined for DIM after 4 weeks of twice-daily 400-mg doses of I3C are only 13% higher than the corresponding values for a single dose of 400 mg, indicating no alteration in kinetics from the single-dose case. This four-week I3C treatment at 400 mg twice daily resulted in a marked induction of CYP1A2, and in a doubling of the urinary 2-hydroxyestrone:16α-hydroxyestrone ratio (14). Moreover, the change in the estrone hydroxylation ratio was obtained after four weeks at 200 mg I3C twice daily. Both of these changes elicited by I3C treatment fit with proposed mechanisms of chemoprevention by this agent, and if DIM is the active species eliciting these changes then we also have a target plasma concentration and AUC for chemoprevention. Our current findings with BR-DIM show that this

target C_{max} would be obtained at a single dose of less than 150 mg, and that the target AUC would be achieved from a single dose between 150 and 200 mg. Based on this analysis, we are currently carrying out a multiple-dose study with BR-DIM at doses of 100 and 200 mg administered twice daily. This study will assess the influence of BR-DIM on the activity of multiple hepatic enzymes including CYP1A2, CYP3A4, CYP2C9, and CYP2D6.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

1. Wattenberg LW, Loub WD. Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. Cancer Res 1978;38:1410–3.
2. Nixon JE, Hendricks JD, Pawlowski NE, et al. Inhibition of aflatoxin B_1 carcinogenesis in rainbow trout by flavone and indole compounds. Carcinogenesis 1984;5:615–9.
3. Fong AT, Hendricks JD, Dashwood RH, et al. Modulation of diethylnitrosamine-induced hepatocarcinogenesis and O^6-ethylguanine formation in rainbow trout by indole-3-carbinol, α-naphthoflavone, and Aroclor 1254. Toxicol Appl Pharmacol 1988;96:93–100.
4. Bradlow HL, Michnovicz J, Telang NT, Osborne MP. Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. Carcinogenesis 1991;12:1571–4.
5. Chung FL, Morse MA, Eklind KI, Xu Y. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis by compounds derived from cruciferous vegetables and green tea. Ann NY Acad Sci 1993;686:186–201.
6. Kojima T, Tanaka T, Mori H. Chemoprevention of spontaneous endometrial cancer in female donryu rats by dietary indole-3-carbinol. Cancer Res 1994;54:1446–9.
7. Grubbs CJ, Steele VE, Casebolt T, et al. Chemoprevention of chemically-induced mammary carcinogenesis by indole-3-carbinol. Anticancer Res 1995;15:709–16.
8. El Bayoumy K, Upadhyaya P, Desai DH, et al. Effects of 1,4-phenylenebis (methylene)selenocyanate, phenethyl isothiocyanate, indole-3-carbinol, and *d*-limonene individually and in combination on the tumorigenicity of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. Anticancer Res 1996;16:2709–12.
9. Jin L, Qi M, Chen D-Z, Anderson A, et al. Indole-3-carbinol prevents cervical cancer in human papilloma virus type 16 (HP V16) transgenic mice. Cancer Res 1999;59:3991–7.
10. Mori H, Sugie S, Rahman W, Suzui N. Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5]pyridine-induced mammary carcinogenesis in rats. Cancer Lett 1999;143:195–8.
11. Verhoeven DTH, Goldbohm RA, van Poppel G, et al. Epidemiological studies on Brassica vegetables and cancer risk. Cancer Epidemiol Biomarkers Prev 1996;5:733–48.
12. Keck A-S, Finley JW. Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. Integr Cancer Ther 2004;3:5–12.
13. Reed GA, Arneson DW, Putnam W III, et al. Single- and multiple-dose administration of indole-3-carbinol to women: pharmacokinetics based on 3,3′-diindolylmethane. Cancer Epidemiol Biomarkers Prev 2006;15:2477–81.
14. Reed GA, Peterson KS, Smith HJ, et al. A phase I study of indole-3-carbinol in women: tolerability and effects. Cancer Epidemiol Biomarkers Prev 2005;14:1953–60.
15. Stresser DM, Bjeldanes LF, Bailey GS, Williams DE. The anticarcinogen 3,3′-diindolylmethane is an inhibitor of cytochrome P-450. J Biochem Toxicol 1995;10:191–201.
16. Le HT, Schaldach CM, Firestone GL, Bjeldanes LF. Plant-derived 3,3′-diindolylmethane is a strong androgen antagonist in human prostate cancer cells. J Biol Chem 2003;278:21136–45.
17. Dalessandri KM, Firestone GL, Fitch MD, et al. Pilot study: effect of 3,3′-diindolylmethane supplements on urinary hormone metabolites in postmenopausal women with a history of early-stage breast cancer. Nutr Cancer 2004;50:161–7.
18. Leibelt DA, Hedstrom OR, Fischer KA, et al. Evaluation of chronic dietary exposure to indole-3-carbinol and absorption-enhanced 3,3′-diindolylmethane in Sprague-Dawley rats. Toxicol Sci 2003;74:10–21.
19. Anderton MJ, Manson MM, Verschoyle R, et al. Physiological modeling of formulated and crystalline 3,3′-diindolylmethane pharmacokinetics following oral administration in mice. Drug Metab Dispos 2004;32:632–8.
20. Zeligs MA, Jacobs IC. Compositions and methods of adjusting steroid hormone metabolism through phytochemicals. U.S. patent 6,086,915, 2000.
21. Kim DJ, Han BS, Ahn B, et al. Enhancement by indole-3-carbinol of liver and thyroid gland neoplastic development in a rat medium-term multiorgan carcinogenesis model. Carcinogenesis 1997;18:377–81.
22. Xu M, Orner GA, Bailey GS, Stoner GD, Horio DT, Dashwood RH. Post-initiation effects of chlorophyllin and indole-3-carbinol in rats given 1,2-dimethylhydrazine or 2-amino-3-methylimidazo[4, 5-f]quinoline. Carcinogenesis 2001;22:309–14.
23. Yoshida M, Katashima S, Ando J, et al. Dietary indole-3-carbinol promotes endometrial adenocarcinoma development in rats initiated with *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine, with induction of cytochrome P450s in the liver and consequent modulation of estrogen metabolism. Carcinogenesis 2004;25:2257–64.
24. Tilton SC, Hendricks JD, Orner GA, Pereira CB, Bailey GS, Williams DE. Gene expression analysis during tumor enhancement by the dietary phytochemical, 3,3′-diindolylmethane, in rainbow trout. Carcinogenesis 2007;28:1589–98.
25. Crowell JA, Page JG, Levine BS, Tomlinson MJ, Hebert CD. Indole-3-carbinol, but not its major digestive product 3,3′-diindolylmethane, induces reversible hepatocyte hypertrophy and cytochromes P450. Toxicol Appl Pharmacol 2006;211:115–23.
26. Grose KR, Bjeldanes LF. Oligomerization of indole-3-carbinol in aqueous acid. Chem Res Toxicol 1992;5:188–93.
27. Staub RE, Onisko B, Bjeldanes LF. Fate of 3,3′-diindolylmethane in cultured MCF-7 human breast cancer cells. Chem Res Toxicol 2006;19:436–42.
28. Anderton MJ, Manson MM, Verschoyle RD, et al. Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. Clin Cancer Res 2004;10:5233–41.

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Cancer Epidemiol Biomarkers Prev 2008;17:2619-2624.