Enhanced Oral Bioavailability of 3,3'-Diindolylmethane Administered in a Self-Microemulsifying Drug Delivery System (SMEDDS)

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ABSTRACT

3,3'-diindolylmethane (DIM) is a stable, water-insoluble indole that demonstrates chemopreventive activity in several preclinical models for human cancer. Although clinical data have shown that the safety profile of absorption-enhanced, microencapsulated DIM is consistent with its use in cancer chemoprevention in humans, the *in vivo* biological activity of DIM is limited by poor solubility and very low oral bioavailability. Self-Microemulsifying Drug Delivery Systems (SMEDDS) provide a novel approach to improving the systemic delivery of lipophilic agents with poor oral bioavailability. Lipid-based SMEDDS formulations form oil-in-water microemulsions in aqueous gastric fluid; these microemulsions increase drug solubility and improve systemic absorption. The present study was performed to determine if the oral bioavailability of DIM and its levels in the prostate are increased by administration in a DIM-specific SMEDDS formulation. Fifteen male CD rats per group received a single oral (gavage) dose of either a microencapsulated DIM formulation (BR-DIM®, BioResponse) or a SMEDDS formulation of DIM (BR-9001, BioResponse). Both groups received a DIM-equivalent dose of 30 mg/kg. Gavage administration of each DIM formulation was immediately followed by a second gavage dose of saline to provide uniform intragastric emulsification of BR-9001 and suspension of BR-DIM. Cohorts of three rats per group were bled at time 0 (pre-dose) and at 0.25, 0.5, 0.75, 1.0, 2.0, 4.0, 8.0, and 24 hours post-dosing; each rat was bled twice. After the second bleed, each rat was euthanized and its prostate was collected. Plasma samples and weighed samples of prostate were extracted and analyzed for DIM content by LC-MS/MS; pharmacokinetics (PK) parameters were calculated using WinNonlin software. The oral bioavailability of DIM was substantially greater in the SMEDDS BR-9001 formulation than in the BR-DIM formulation: at the Cmax (30 min), mean plasma DIM levels in rats receiving the SMEDDS formulation were >400% of mean plasma DIM levels in rats dosed with microencapsulated BR-DIM. For four hours post-dosing, plasma DIM levels in SMEDDS-treated rats remained above plasma DIM levels in rats treated with microencapsulated DIM; the AUC for the SMEDDS formulation of DIM was approximately twice that of microencapsulated DIM. Similarly, at 2, 4, and 6 hours post-dosing, mean prostate DIM levels in rats receiving the SMEDDS formulation were approximately 200% of mean prostate DIM levels in rats receiving microencapsulated BR-DIM. These data demonstrate that plasma and prostate levels of DIM can be increased by using the BR-9001 SMEDDS formulation of DIM. Because the chemopreventive efficacy of DIM appears to be limited by poor oral bioavailability, the use of SMEDDS formulation technology may increase the efficacy of DIM for cancer chemoprevention.

BACKGROUND AND INTRODUCTION

Indole-3-carbinol (I3C) and its condensation product, diindolylmethane (DIM) both demonstrate significant cancer preventive activity in animal models, and may inhibit both the initiation and promotion/progression phases of carcinogenesis. Inhibition of tumor initiation by indoles appears to be mediated by inhibition of several cytochrome P450s; indoles also modulate the activity of glutathione S-transferase and other Phase II enzymes, and may thereby enhance carcinogen detoxification and excretion.

Other biological activities of I3C and DIM suggest potential efficacy in inhibiting tumor promotion and progression. These include: inhibition of cell proliferation, induction of apoptosis, inhibition of inflammation, and inhibition of angiogenesis.

DIM and Prostate Cancer Prevention: Experimental Data

Experimental data suggest that DIM may be an effective agent for prostate cancer prevention in humans. DIM demonstrates antiandrogenic activity in the prostate that is mediated by downregulation of the androgen receptor (AR) and/or inhibition of AR translocation to the nucleus. *In vivo*, DIM inhibits prostate cancer development in the TRAMP mouse and the growth of prostate cancer xenografts in athymic mice. *In vitro*, DIM inhibits proliferation and induces apoptosis in several prostate cancer cell lines; mechanisms proposed for these activities include down-regulation of NF-κB, Nrf2, Akt, and/or AR-dependent signaling pathways.

DIM and Prostate Cancer Prevention: Clinical Data

Several clinical trials have been conducted to evaluate the tolerability and safety of DIM in men with or at high risk for prostate cancer. In a pre-surgical protocol, short-term administration of DIM (100 or 200 mg BID) was well-tolerated in 45 patients with prostate cancer. Similarly, daily oral administration of 900 mg DIM for three months was well-tolerated by men with prostatic intraepithelial neoplasia (PIN).

Although DIM was well-tolerated in both trials, its oral bioavailability is quite low. For this reason, recent studies have focused on absorption-enhanced DIM formulations that demonstrate improved oral bioavailability. In a Phase 1 dose escalation trial with BR-DIM (a microencapsulated formulation of DIM used as the comparator in the present study), oral doses of up to 300 mg DIM equivalent/day were well-tolerated by men with castration-resistant prostate cancer. Clinical proof-of-concept was provided in a randomized, placebo-controlled clinical trial in which administration of a DIM formulation with improved oral bioavailability for one year induced a statistically significant reduction in the severity of PIN lesions.

Enhancement of the Oral Bioavailability of DIM using Self-Emulsifying Drug Delivery Systems (SMEDDS)

DIM demonstrates potent antiproliferative, pro-apoptotic, and other desirable activities in cancer cells *in vitro* and has significant chemopreventive activity when administered at high doses in carcinogenesis studies in animals. However, the clinical efficacy of DIM for prostate cancer prevention is likely to be limited by poor oral bioavailability resulting from its limited solubility.

Self-Emulsifying Drug Delivery Systems (SMEDDS) are composed of oil, a surfactant, and a co-surfactant, and provide a novel approach to increase the oral bioavailability of poorly soluble drugs. During normal gastric action, SMEDDS formulations mix with water to form oil-in-water microemulsions that "trap" drug at the oil/water interface. The large surface area of the oil/water interface within the microemulsion provides an effective means to increase delivery of a poorly soluble compound to the intestinal epithelium without requiring its dissolution. Administration of poorly soluble drugs in SMEDDS can increase drug bioavailability, and several SMEDDS formulations have been approved by the FDA for clinical use.

RATIONALE

Although the biological activities of DIM are consistent with efficacy in prostate cancer prevention, *in vivo* efficacy may be limited by poor oral bioavailability. Development of DIM formulations with increased oral bioavailability is a critical step in the advanced preclinical and clinical development of DIM.

Absorption-enhanced formulations (e.g., BR-DIM, a microencapsulated formulation of DIM) can improve oral bioavailability by up to five-fold over unformulated crystalline DIM; this greater oral bioavailability may greatly increase efficacy in prostate cancer prevention. The present study was performed to compare the oral bioavailability and tissue distribution of BR-DIM (a microencapsulated DIM formulation) with that of BR-9001, a self-microemulsifying drug delivery system (SMEDDS) formulation of DIM.

MATERIALS AND METHODS

Animal Welfare: Prior to the initiation of experimentation, the study protocol was reviewed and approved by the IIT Research Institute Animal Care and Use Committee. All work was performed in full compliance with NIH Guidelines for the Care and Use of Laboratory Animals.

Study Design: The study design is summarized in Table 1.

Table 1: Comparative Oral Bioavailability and PK of BR-9001/BR-DIM in Rats

Group	Treatment	Type of DIM Formulation	Dose Level (mg DIM/kg/dose)	No. of Doses	No. of Male Rats
1	BR-9001	SMEDDS	30	1	15
2	BR-DIM	Microencapsulated	30	1	15
3	BR-9001	SMEDDS	30	3	3
4	BR-DIM	Microencapsulated	30	3	3

- Rats in Groups 1 and 2 received a single oral (gavage) dose of BR-9001 (microencapsulated DIM, BioResponse, Boulder CO) or BR-DIM (SMEDDS formulation of DIM, BioResponse), immediately followed by a single oral (gavage) dose of 0.9% NaCl ("saline chaser"). The saline chaser was administered to facilitate microemulsification of BR-9001 in the stomach. Both test articles and the saline chaser were administered at dosing volumes of 5 mL/kg.
- Each group of 15 rats was divided into five cohorts of 3 rats each for timed bleeds for plasma drug level analyses. Each rat was bled twice after dosing, using the following schedule:
  - Cohort 1: bled at pre-dose (0 hour) and 2 hours post-dose on Day 1
  - Cohort 2: bled at 0.25 and 4 hours post-dose on Day 1
  - Cohort 3: bled at 0.5 and 6 hours post-dose on Day 1
  - Cohort 4: bled at 0.75 and 8 hours post-dose on Day 1
  - Cohort 5: bled at 1 hour post-dose on Day 1 and 24 hours post-dose (on Day 2)
- Rats in Groups 3 and 4 each received three oral (gavage) doses of either BR-9001 or BR-DIM (immediately followed by "saline chaser") at intervals of 12 hours. Each rat in Groups 3 and 4 was bled at 1 hour after the final dose of BR-9001 or BR-DIM.
- After its last scheduled blood collection, each rat was immediately euthanized, prostates were removed *en bloc*, and prostate lobes were dissected out. Prostates were gently blotted to remove fluid, weighed, snap frozen, and stored at -70 °C until analyzed for DIM content.

Experimental Animals: Male CD rats (aged 5 to 6 weeks), obtained from Charles River Laboratories, Kingston, NY. Rats were quarantined for 1 week prior to initiation of dosing.

Animal Husbandry:

- Housing: Singly in suspended stainless steel cages during exposure and recovery periods
- Diet: Certified Global 18% Protein Rodent Diet #2018C (Harlan/Teklad, Madison, WI)
- Drinking Water: City of Chicago, supplied by automatic watering system
- Animal Room Light Cycle: 12 hours light/12 hours dark per day

Table 2: HPLC Conditions Used for DIM Analysis

HPLC Column	Polar RP, 30 mm x 2.1 mm , 4 µm (Phenomenex)		
Column Temperature	25°C		
Injection Volume	5 µL		
Flow Rate	0.3 mL/min		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in acetonitrile		
Program	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	70	30
	0.10	70	30
	1.00	5	95
	4.50	5	95
	4.70	70	30
	8.00	70	30
Retention Time	DIM – approximately 4.0 minutes		
Run Time	8 minutes		

Analysis of DIM in Plasma and Prostate: After sample extraction, centrifugation, and dilution, levels of DIM in plasma and prostate were quantitated using an AB SCIEX Model 4000 QTrap LC-MS/MS equipped with an Agilent Model 1200 HPLC.

Table 3: MS/MS Conditions used for DIM Analysis

Scan Type	MRM
Ion Source	Turbo Spray ESI
Ion Spray Voltage	5500 Volts
Polarity	Positive
Ion Source Temperature	725°C
Collision Energy	20 Volts
Ions monitored (Q1®Q3)	247.1 → 130.1
Resolution	Unit
Data System	Analyst® 1.6.3 (Applied Biosystems/MDS Sciex

RESULTS

Toxicity: No drug-associated mortality or clinical evidence of toxicity was seen in any animal exposed to either BR-DIM or BR-9001.

Plasma Drug Levels and Pharmacokinetics (Single Dose):

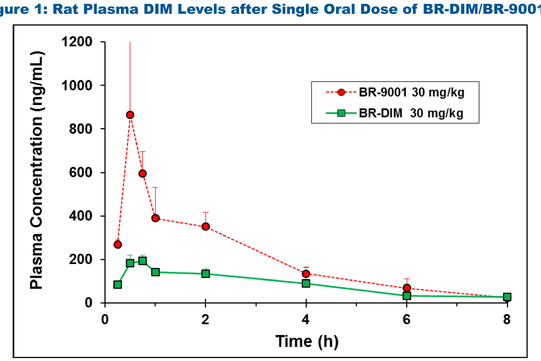
PK parameters determined after oral administration of single equivalent doses of DIM in the SMEDDS formulation (BR-9001) and as BR-DIM are provided in Table 4. Plasma clearance curves after administration of single doses of BR-9001 and BR-DIM are provided in Figure 1.

Table 4: Plasma PK Parameters After Oral Administration of DIM Formulations

Group	Test Article	DIM Dose (mg/kg)	No. of Doses	PK Parameter						
				Rsq	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (hr*ng/mL)	V_d/F (mL/kg)	CL/F (mL/hr/kg)
1	BR-9001	30	1	0.992	1.55	0.50	864	1812	39892	17822
2	BR-DIM	30	1	0.940	2.59	0.75	192	892	144488	38627

- The plasma Cmax after administration of DIM in the SMEDDS formulation (BR-9001) was more than 4X the Cmax after administration of an equivalent dose of DIM as BR-DIM.
- The AUC for DIM after administration of BR-9001 was approximately twice for DIM after administration of BR-DIM.
- The $T_{1/2}$ for plasma DIM after administration of BR-9001 was approximately 1.5 hours, versus approximately 2.5 hours after administration of BR-DIM.

Figure 1: Rat Plasma DIM Levels after Single Oral Dose of BR-DIM/BR-9001



- Absorption of DIM was rapid after gavage exposure to either BR-9001 or BR-DIM. The Tmax for DIM was < 1 hour after administration of both formulations.
- For the first two hours after dosing, plasma DIM levels in rats treated with BR-9001 were 2.5X to 4X plasma levels in rats receiving an equivalent dose of DIM as BR-DIM.
- At 4 and 6 hours, plasma DIM levels in rats treated with BR-9001 were 1.5X to 2X plasma levels in rats receiving an equivalent dose of DIM as BR-DIM.
- At 8 and 24 hours, plasma DIM levels were comparable in the two groups.

Plasma Drug Levels (Three Doses):

Plasma DIM levels at 1 hour after oral administration of the last of three doses of DIM (at 12-hour intervals) in the SMEDDS formulation (BR-9001) and as BR-DIM are provided in Table 5.

Table 5: Plasma DIM Levels after Three Doses of BR-9001 and BR-DIM

Group	Test Article	DIM Dose (mg/kg)	No. of Doses	Plasma Drug Levels (ng/mL)
1	BR-9001	30	3	209 ± 94
2	BR-DIM	30	3	112 ± 24

- Plasma DIM levels in rats receiving 3 oral doses of BR-9001 were ~2X plasma levels in rats receiving 3 equivalent doses of DIM as BR-DIM.

Prostate Drug Levels (Single Dose):

Levels of DIM in the prostate after oral administration of equivalent doses of DIM in the SMEDDS formulation (BR-9001) and as BR-DIM are provided in Table 6.

Table 6: DIM Levels in Prostate after Administration of BR-9001 and BR-DIM

Group	Test Article	Prostate DIM Levels (ng/g)				
		Time Post-Dosing (hours; n = 3/cohort)				
		2	4	6	8	24
1	BR-9001	476 ± 339	164 ± 79	101 ± 48	40 ± 23	BLQ
2	BR-DIM	201 ± 145	101± 52	35 ± 15	35 ± 9	BLQ

- For the first six hours after dosing, levels of DIM in the prostate of rats treated with BR-9001 ranged from 1.5 to 3X levels of DIM in the prostate of rats treated with BR-DIM
- Prostate levels of DIM were comparable in BR-9001 and BR-DIM-treated rats at 8 hours after dosing, and were below the limit of quantitation (0.5 ng/g) in both groups at 24 hours.

Prostate Drug Levels (Three Doses):

DIM levels in the prostate quantitated at 1 hour after administration of the last of three oral doses of BR-9001 or BR-DIM are provided in Table 7.

Table 7: DIM Levels in Prostate After Three Doses of BR-9001 and BR-DIM

Group	Test Article	DIM Dose (mg/kg)	No. of Doses	Prostate Drug Levels (ng/g)
1	BR-9001	30	3	163 ± 44
2	BR-DIM	30	3	186 ± 133

- Prostate levels of DIM were comparable in rats receiving three doses of BR-9001 or BR-DIM (at 12 hour intervals).

CONCLUSIONS

1. DIM was rapidly absorbed after oral (gavage) administration of either BR-9001 (SMEDDS formulation of DIM) or BR-DIM (microencapsulated DIM). Plasma Tmax was < 1 hour for both formulations. The AUC of DIM in rats receiving BR-DIM at 30 mg/kg (DIM equivalent dose) is similar to the AUC of DIM in humans receiving BR-DIM at a DIM equivalent dose of 4 mg/kg.
2. Between 0.25 and 6 hours after a single oral dose, plasma DIM levels in rats treated with BR-9001 were 1.5X to 4X plasma DIM levels measured in rats treated with an equivalent dose of BR-DIM. Similarly, plasma DIM levels after administration of three doses of BR-9001 at 12 hour intervals were approximately 2X plasma DIM levels after administration of BR-DIM by the same schedule. These data clearly demonstrate the improved oral bioavailability of DIM administered in a SMEDDS formulation.
3. Between 2 and 6 hours after dosing, prostate levels of DIM in rats treated with BR-9001 were 1.5X to 3X the prostate levels of DIM measured in rats treated with BR-DIM. However, prostate levels were comparable at 8 and 24 hours after a single dose of the two formulations, and at 1 hour after three doses.
4. These data clearly demonstrate the improved oral bioavailability of DIM administered in a SMEDDS formulation versus an absorption-enhanced microencapsulated formulation of DIM. However, plasma and tissue drug level data at 8 and 24 hours suggest that a minimum of two doses per day are required to maintain prostate DIM concentrations at levels that are likely to be required for cancer preventive activity.

ACKNOWLEDGEMENT

This research was supported by contract HHSN2612015000241 from the Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute.