Pharm Res (2015) 32:2292–2300
DOI 10.1007/s11095-015-1620-7

RESEARCH PAPER

Evaluation of Spray BIO-Max DIM-P in Dogs for Oral Bioavailability and in Nu/nu Mice Bearing Orthotopic/Metastatic Lung Tumor Models for Anticancer Activity

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Received: 23 September 2014 / Accepted: 5 January 2015 / Published online: 10 January 2015

ABSTRACT

Purpose In an effort to prepare an oral dosage form for poorly bioavailable anti-cancer agents, we have incorporated spray drying using a customized spray gun generating enteric coated Self-emulsifying drug delivery systems. The objective of this study was to design and evaluate pharmacokinetics and pharmacodynamic characteristics of Spray BIO-Max DIM-P (SB DIM-P).

Methods SB DIM-P was prepared and optimized based on physico-chemical characteristics using design of experiment (DOE–Vr 8.0) software. Pharmacokinetic parameters in dogs and rats were evaluated and analyzed using Winonlin. Anti-tumor activity was carried out in orthotopic and metastatic lung tumor models using size M capsules in mice.

Results Based on the optimization using DOE analysis of SB DIM-P characteristics, formulations were selected for further investigation. Pharmacokinetic studies showed a 30% increase in oral bioavailability in rats and ~2.9 times more bioavailability of SB DIM-P compare to solution in dogs. SB DIM-P showed ~20–25% more tumor volume/weight reduction in H1650 metastatic tumor model and ~25–30% tumor volume/weight reduction in A549 orthotopic tumor model compared to DIM-P solution.

Conclusions Our studies demonstrate the potential application of spray dried enteric coated self-emulsifying delivery system (SB DIM-P) to enhances oral absorption and efficacy of DIM-P in lung tumor models.

KEY WORDS anti-cancer activity · DIM · dual channel spray drying · enteric coating · self-emulsifying drug delivery

ABBREVIATIONS

AUC	Area under the curve
C-DIMs	C substituted DIM desrivatives
Cmax	maximum serum concentration
DIM	Di-indole-methane
DIM-P	1, 1-bis (3′-indolyl)-1-(p-substituted phenyl) methanes
DOE	Design of experiment
i.v.	intravenous
PAMPA	Parallel artificial membrane permeability assay
PPARγ	Peroxisome proliferator activated receptor γ
RSM	Response surface methodology
SB DIM-P	Spray BIO-Max DIM-P (spray dried enteric coated self-emulsified drug delivery system for DIM-P)
SD	Standard deviation
SE	Self emulsify
SEDDS	Self-emulsifying drug delivery systems
S-SEDDS	Solid self-emulsified drug delivery systems
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
VEGF	Vascular endothelial growth factor

INTRODUCTION

Approximately 40% of new active pharmaceutical agents are associated with low oral bioavailability due to poor aqueous solubility (1). Our laboratory has reported the promising anticancer activity of 1, 1-bis (3′-indolyl)-1-(p-substituted phenyl) methanes (DIM-P) [C-substituted di-indole-methane derivative (DIMs)] (2) in lung cancer models by inhalation and oral

Electronic supplementary material The online version of this article (doi:10.1007/s11095-015-1620-7) contains supplementary material, which is available to authorized users.

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routes of administration (3,4). DIM-P in combination with docetaxel showed moderate synergistic anticancer activity in lung tumor cell lines in vitro and additive effect in vivo. Further, even though DIM-P is a desirable anticancer agent, its activity as a single agent is limited due to its poor oral bioavailability which is only 13% (2).

Various formulation strategies which can be explored to enhance oral bioavailability of poorly soluble drugs. Such as, use of surfactants/lipids/permeation enhancers, β-cyclodextrins complexes, salt formation, micronization, nanocarriers, solid dispersions, spray drying and self-emulsifying drug delivery systems (SEDDS) (1). SEDDS are mixtures of oils, surfactants, solvents and co-solvents that creates oil-in-water emulsions upon mild stirring in water. SEDDS offer several advantages which includes their spontaneous formation, thermodynamic stability, improved bioavailability (5) and ease of manufacturing. Following their oral administration, these systems rapidly disperse in gastrointestinal fluids to yield micro or nano emulsions and are rapidly absorbed through the lymphatic pathway (6). Various bioavailability studies have reported that lipophilic compounds, such as, simvastatin and halofantrine, are more efficiently up taken from gastrointestinal tract when administered in SEDDS (7–9). Nevertheless, SEDDS which are usually prepared as liquid dosage forms pose few disadvantages; low drug loading, high production costs, few choices of dosage forms, portability and possible gastrointestinal irritation due to higher quantities of surfactants used in formulations. Thus, solid self-emulsified drug delivery systems (S-SEDDS) were developed as an alternative (10) and they involve the solidification of liquid self-emulsifying (SE) ingredients into solid products which could be nano or micro particles (11) and can be marketed as tablets, pellets and capsules (12,13).

Spray drying is a relatively simple, cost-effective and scalable technology that has been used to produce particles even with labile compounds (14). Polymers commonly employed in the spray drying process are methacrylic copolymers, polyesters, chitosan and alginates (15,16) and these have been used for a variety of drugs including chemotherapeutics, antibiotics, and anti-inflammatory agents (14,17–19). Similarly, it's been used to fabricate polymeric micro particles for systemic and controlled delivery of various bioactive pharmaceutical materials with poor pharmacokinetic or toxicity concerns (12). Our laboratory has designed a custom made dual channel spray drying gun technology to formulate solid form of SEEDS for lipophilic drugs. The unique nature of our dual channel spray gun enables the spray drying of two different liquid systems (e.g lipid mixture containing the drug (DIM-P) and polymer coating) simultaneously so that a uniform coating is obtained on the micro particles. DIM-P stability and pharmacokinetic studies conducted in our laboratory demonstrated that about 20% of the total administered drug is degraded in the stomach. Thus we have used here enteric coating with the S-SEDDS to improve bioavailability of DIM-P. It is expected that the use of our dual channel spray drying technique will lead to a superior formulation of the DIM-P (SB DIM-P) which will have higher bioavailability and also enhanced pharmacodynamic activity when evaluated in lung cancer models. The objective of this study was to design and evaluate "Spray BIO-Max (SB DIM-P)" (spray dried enteric coated self emulsified drug delivery system for DIM-P) in dogs and rats for pharmacokinetic analysis and in mice for anti-cancer activity against A549 orthotopic and H1650 metastatic lung cancer models.

MATERIALS AND METHODS

Materials

DIM-P was prepared as described (20). A549, H1650 and Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD, USA). Cell culture were grown in F12K, RPMI 1640 and DMEM medium with 10% fetal bovine serum obtained from Invitrogen (Grand Island, NY, USA) and antibiotic-antimycotic solution PSN mix by Gibco-Invitrogrn (Grand Island, NY, USA). The cells were maintained at 37°C in the presence of 5% CO2. All other chemicals used were of analytical grade.

Animals

Male Sprague-Dawley rats (240 to 350 g) and Nu/nu mice (20–30 g) were used. The protocols were approved by the Institutional Animal Care and Use Committee, Florida A & M University. Animals were given standard animal diet, in a controlled room (22 ± 1°C @ 35–50% RH) for a week prior to experiments. Eighteen month old female intact Labrador retriever dogs were acquired from an internal canine breeding colony maintained at Texas A&M, College of Veterinary Medicine. All canine protocols were approved by the Institutional Animal Care and Use Committee at Texas A&M University. Dogs were housed in large runs and allowed outdoor play time and toys for enrichment. They were fed standard dog chow and water *ad libitum* for the duration of the study.

Preparation of Liquid Self-Emulsifying System and SB DIM-P

The liquid self-emulsified (SE) formulations were prepared as previously reported (21). Initially, solubility of DIM-P was determined in different oils and surfactants to select the suitable oil to be used for the formulation. The mixture of oils, surfactants and co-surfactants was optimized by DOE

analysis. Briefly, the DIM-P was dissolved into the mixture of oil, surfactant, and co surfactant with help of heating at 50°C in a water bath and vortexed until a clear solution was obtained. Then it was kept at room temperature for 24 h and examined for stability parameters such as turbidity/phase separation. A self-emulsified DIM-P formulation in liquid form was used for the spray drying. To prevent the gastric degradation of the drug, the dried particles were enteric coated. The dual channel spray drying system (Fig. S1) termed as S*ingle Spray Gun* is modified from conventional single channel, where only one liquid may be spray dried. This modification allows us to spray two separate liquid systems containing one or more active pharmaceutical agent(s). Multi-layered microstructures prepared by dual channel (spray gun) spray dryer which enables simultaneous drying of inner core or droplets embedded into outer layer or matrix of excipients to enable various combination of formulation to enhance active pharmaceutical agents bioavaibility by enhanced absorption in the gastrointestinal tract. Also, this technology reduces the steps involved in conventional formulation design [e.g., enteric coated formulation requires two steps conventionally: 1) formulation of particle/tablet etc, 2) followed with coating] and produces fine, uniform product in single step as we have shown here for enteric coated self-emulsified formulation for DIM-P. Various solutions and steps involved in the preparation of SB DIM-P are as follows. Solution 1: solution consists of DIM-P self-emulsified formulation prepared as described above. Solution 2: The polymer solutions to provide enteric coating.

Quality by Design—Response Surface Methodology (RSM) and Desirability Function

A Quality by Design concept was used to create different SEDDS and SB DIM-P formulations utilizing oil, surfactant, and co-surfactant by dissolving DIM-P in oil. Response surface designs was used to discover variables that offers process and product improvement in the experimental region using polynomial equation (2). The objective was to select the formulation composition for SB DIM-P which could improve bioavailability significantly compared to that of free drug. All experimental results were calculated using statistical software, DOE v6.0.5 (Stat-Ease, Inc., Minneapolis, MN). The multiple response method utilizes the desirability function, which reflects the desired outcome for each response (2). At a given point in the experimental domain the desirability can be calculated where optimum is the point with the highest value for the desirability. The particle/droplet size and drug release was optimized since this affects absorption of the drug in gastrointestinal tract.

Please refer to Supplementary data section (Material and Methods) for Characterization of self-emulsified spray dried formulations, Preparation and Evaluation of Capsules for Mice and Parallel artificial membrane permeability assay (PAMPA) of DIM-P and SB DIM-P.

Pharmacokinetic Analysis of DIM-P and SB DIM-P

A) Pharmacokinetics in Rat: Pharmacokinetic parameters were determined as described earlier (2). Animals were randomly divided ($n=5$) and treatment groups were given 20 mg/kg of equivalent DIM-P orally. The third group received i.v. injection into the tail vein (5.0 mg/kg). Blood samples were collected at predetermined time points: 0.017 (only following IV dosing), 0.25, 0.5, 0.75, 1, 3, 6, 8 and 24 h. Plasma were separated using centrifugation and stored at –80°C until HPLC analysis.

B) Pharmacokinetics in Dogs: Pharmacokinetic profile of DIM-P in Dogs was determined following IV and oral administration groups ($n=3$). DIM-P was formulated as described earlier for intravenous administration and for oral solution; DIM-P was dissolved in corn oil. The oral treatment groups were given 3.33 mg/kg of DIM-P solution and SB DIM-P equivalent to 3.33 mg/kg of DIM-P was administered orally by syringe. Each dog had a central venous catheter (long saphenous) placed on the day of the study. The third group was given DIM-P (0.5 mg/kg) intravenously. Dogs were fasted overnight before the start of pharmacokinetic studies. Blood samples were collected at baseline, and at 15, 30, 60, 120, 180, 240, 360, 480, 600, 720 and 1440 min after administration of a single dose of DIM-P solution and SB DIM-P from the venous catheters into heparinized tubes. Blood samples were immediately centrifuged and plasma was collected and stored at −80°C until analysis. At the end of the study, major organs were collected for further evaluation.

C) Pharmacokinetic data analysis: Pharmacokinetic analysis (DIM-P extraction, HPLC analysis and parameters) were determined as described earlier using non-compartmental techniques (2,22,23).

In Vivo Anticancer Evaluation in Lung Cancer Models

A) Orthotopic A549 tumor model: The orthotopic lung cancer model was used to mimic the lung cancer in humans using (female, 6-week old) BALB/C athymic nude mice as described previously (3,4). Mice were randomly divided into groups to receive treatment after 7–10 days of cell inoculation.

B) Metastatic H1650 tumor model: The metastatic H1650 tumor model was developed using Nu/Nu mice as described previously (24,25). Mice were randomly divided into groups to receive treatment after 7–10 days of cell inoculation.

Treatment of Animals

A) Orthotopic A549 tumor model: Seven days after tumor implantation, mice were randomly divided into the following groups ($n=12$) to receive DIM-P formulations by oral gavage. The control group received vehicle (No DIM-P); the second group received DIM-P (20 mg/kg) solution every other day; the third group received SB DIM-P (20 mg/kg).

B) Metastatic H1650 tumor model: Mice were randomly divided into the following groups ($n=12$) to receive treatments. Size M capsules filled with blank formulation and SB DIM-P were delivered according to manufacturer's protocol. The control group received vehicle (No DIM-P); the second group received DIM-P (20 mg/kg) solution every other day and the third group received SB DIM-P (20 mg/kg).

Evaluation of Anti-cancer activity was carried out as mentioned previously in terms of tumor weight/volume (24). Also, TUNEL assay, IHC for VEGF expression and Western blot analysis of lung tumor tissues were carried out as described earlier (23).

Statistical Analysis

Data were expressed as mean ± standard deviations (SD) and model parameters as estimates with ± standard errors and compared using one-way variance analysis (ANOVA); or two-way ANOVA analyses where applicable. Probability (p) values <0.05 were considered significant. All statistical analyses were performed using GraphPad Prism® 5.0 software (San Diego, CA).

RESULTS

Experimental Design and Optimization Using Desirability Function

The SEDDS and SB DIM-P were optimize using Response surface methodology (RSM). Based on the preliminary investigation of variable parameters (particle size and drug release) factors (A), amount of DIM-P, (B) oil and (C) surfactant were further investigated. The mathematical relationship was obtained using DOE v6.0.5 (Stat-Ease, Inc.) to link dependent and independent variables. The conclusions were made using polynomial equations. Multiple linear regression analysis showed that A2 and AB terms were irrelevant for particle size, AC and BC terms were also irrelevant for drug release. The A, B, and C in the equation were obtained by substituting with predicted values, which were in close agreement with experimental values. The contour plots and interactions between independent variables are shown in Fig. S2. A single response was obtained using desirability function by combining all measured responses. A desirability value of 0.81 and 0.97 for SEDDS and SB DIM-P were identified as optimized batch from all measured responses (Tables S1 and S2). The optimized formulation composition is shown in Table S3.

Pharmacokinetic Studies of SB DIM-P

A) Pharmacokinetics in Rats: The oral plasma drug profile of DIM-P at 20 mg/kg dose showed an absence of lag time in the absorption phase (Fig. 1). Oral delivery of DIM-P (20 mg/kg) showed poor bioavailability (<20%) and a shorter plasma half-life compared to that of SB DIM-P (Table S4). The pharmacokinetic profile following i.v. administration of DIM-P (5 mg/kg) showed an initial decline in concentration followed by a slow elimination phase with a plasma half-life of 0.8 h (Table S4). Overall, DIM-P exhibited shorter 0.8 h (i.v.) and a longer 8 h (oral) half-life. However the half-life for SB DIM-P was increased by ~3 h with increased bioavailability. Pharmacokinetic analysis showed an increase in AUC from 62.24 ± 19.84 ug.h/ml to 189.89 ± 62.86 ug.h/ml for DIM-P in solution compare to SB DIM-P respectively. Other pharmacokinetic parameters are shown in Table S4.

B) Pharmacokinetics in Dogs: The plasma concentration–time profiles of DIM-P formulations in dogs are shown in Fig. 2. Non-compartmental pharmacokinetic parameters are shown in Table S5. AUC was calculated by trapezoidal methods ($p<0.05$). Oral delivery of DIM-P (3.33 mg/kg) showed poor bioavailability (<12%) and a shorter plasma half-life compared to that of SB DIM-P (Table S5). However, the half-life for SB DIM-P was increased by ~3 h with increase in bioavailability by ~30%. Pharmacokinetic evaluation in dogs showed improved absorption of SB DIM-P formulations compared to solution; increased Cmax (38.81 ± 6.67 vs 19.26 ± 4.52 μg dL-1) and higher AUC0–t (31216.98 ± 1025.69 vs 10656.25 ± 539.27 μg min dL-1). The relative oral bioavailability of SB DIM-P calculated on the basis of AUC0–t was about 293% more as compared to solution. Other pharmacokinetic parameters are shown in Table S5.

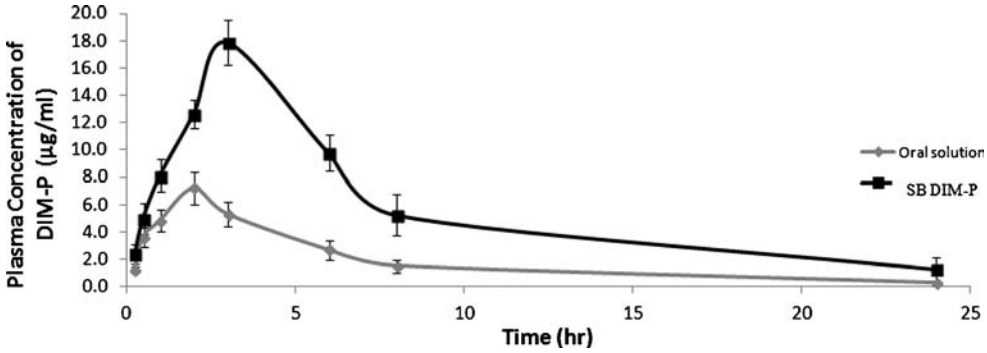

Fig. 1 Plasma concentration (µg/ml) Vs Time profile (hr) following oral administration of DIM-P solution (20 mg/kg) and self-emulsified spray dried formulation of DIM-P (20 mg/kg) (SB DIM-P) in rats ($n=5$).

In Vivo Anticancer Activity of Oral DIM-P

A) Orthotopic A549 tumor model: Seven days after inoculation with tumor cells, the average lung weight and tumor volume were 245 ± 15.89 mg and 215 ± 21.48 mm^3, respectively. Seven days after tumor implantation, treatment was given for a total of 28 days. The lung tumor weights were significantly ($P<0.001$) decreased by 56 and 34% after treatment with SB DIM-P and DIM-P respectively compared to vehicle control (Fig. 3). In mice treated with the SB DIM-P & DIM-P lung tumor volumes were decreased by 66 and 41% respectively. SB DIM-P and DIM-P treatment showed significant ($P<0.001$) decrease in average number of tumor nodules by 56 and 29% respectively compared to control groups.

B) Metastatic H1650 tumor model: Ten days after tumor implantation, treatment was given for a total of 28 days. The lung tumor weights were significantly ($P<0.001$) decreased by 53 and 26% after treatment with SB DIM-P and DIM-P respectively compared to vehicle control (Fig. 4). In mice treated with the SB DIM-P & DIM-P lung tumor volumes were decreased by 56 and 31% respectively. SB DIM-P and DIM-P treatment showed significant ($P<0.001$) decrease in average number of tumor nodules by 49 and 22% respectively compared to control groups.

Molecular Analysis of Lung Tumors

The lung tumor histology was evaluated by H & E staining of lung tumor tissue. DIM-P and SB DIM-P treated tumors exhibited only occasional, isolated microvessels, while tumors from untreated mice had well-formed capillaries surrounding nests of tumor cells. Histological examination of the lung tissue sections showed no signs of inflammation or edema among all groups which suggests a safer toxicity profile for both DIM-P and SB DIM-P therapy. TUNEL assay was carried out on tumor tissue sections for detection of DNA fragmentation. DIM-P induced 22 ± 6% DNA fragmentation (brown staining) whereas 49 ± 7% of tumors from SB DIM-P treated mice showed DNA fragmentation (Fig. S3A). The VEGF expression of was decreased 58 ± 7 and 26 ± 5% after treatment with SB DIM-P and DIM-P respectively compared to no treatment with

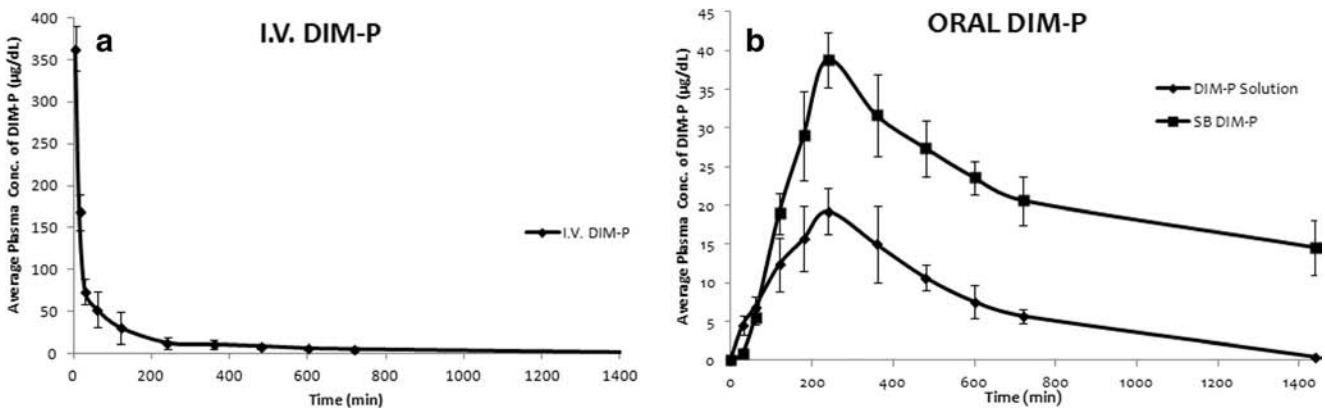

Fig. 2 (**a**) Plasma concentration (µg/ml) Vs Time profile (hr) following intravenous administration of DIM-P (0.5 mg/kg), (**b**) Plasma concentration (µg/ml) Vs Time profile (hr) following oral administration of DIM-P solution (3.33 mg/kg) and self-emulsified spray dried formulation of DIM-P (3.33 mg/kg) (SB DIM-P) in dogs ($n=3$).

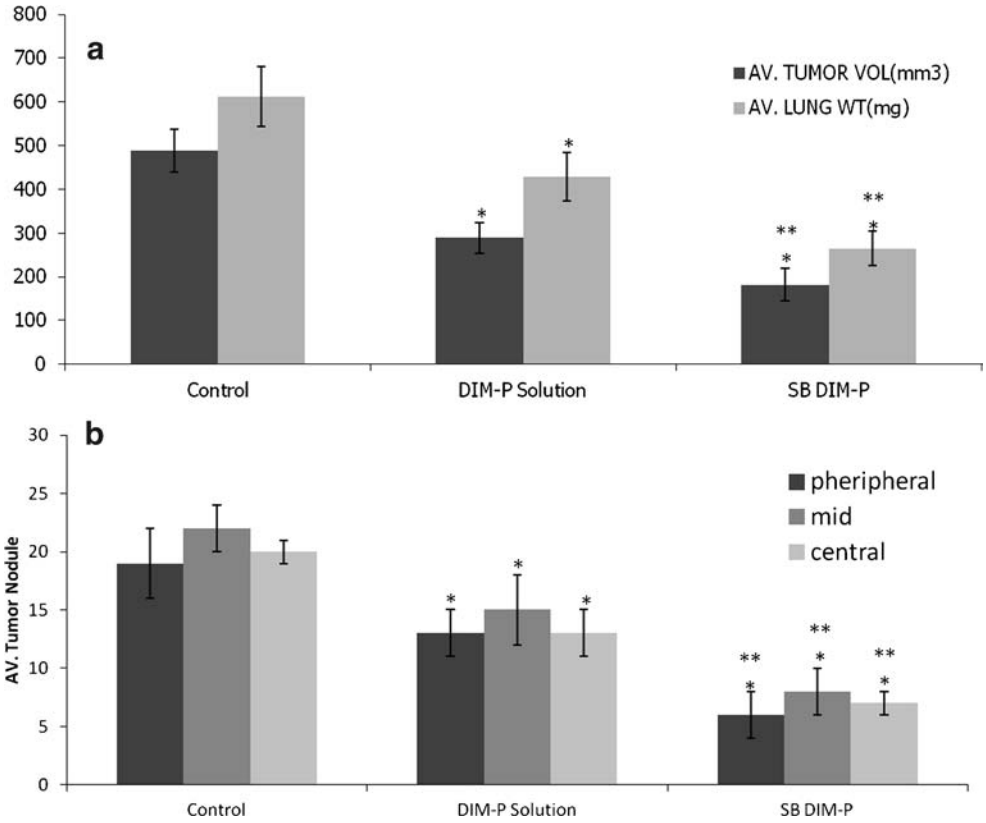

Fig. 3 Effects of DIM-P and SB DIM-P on orthotopic A549 lung tumor weight and tumor volume (**a**); and tumor nodules in central, mid and pheripheral regions of lungs (**b**). A549 cells were injected into the lungs of nude mice. Tumors were established for 7 days before therapy. Tumors from animals treated with DIM-P and SB DIM-P. Lung weights and tumor volumes were determined for measurement of therapeutic activity of the treatments. Tumor nodules of 2–10 mm^3 in volume were counted using harvested lungs for control and treated groups and the average number of tumor nodules were determined. One-way ANOVA followed by post Tukey test was used for statistical analysis. $P < 0.05$ (*Significantly different from untreated controls, **Significantly different from DIM-P solution). Data presented are means ± SD ($n = 12$).

DIM-P (Fig. S3B). Furthermore, SB DIM-P increased phospho-JNK expression significantly ($P<0.05$) to 4.6-fold compared to 1.8-fold with DIM-P ($P<0.01$), respectively of controls in tumors (Fig. S4). The SB DIM-P and DIM-P treatment increased kinases MKK4 and ASK1 expression significantly ($P<0.001$) and this protein was non-detectable in tumors from control mice (Fig. S4).

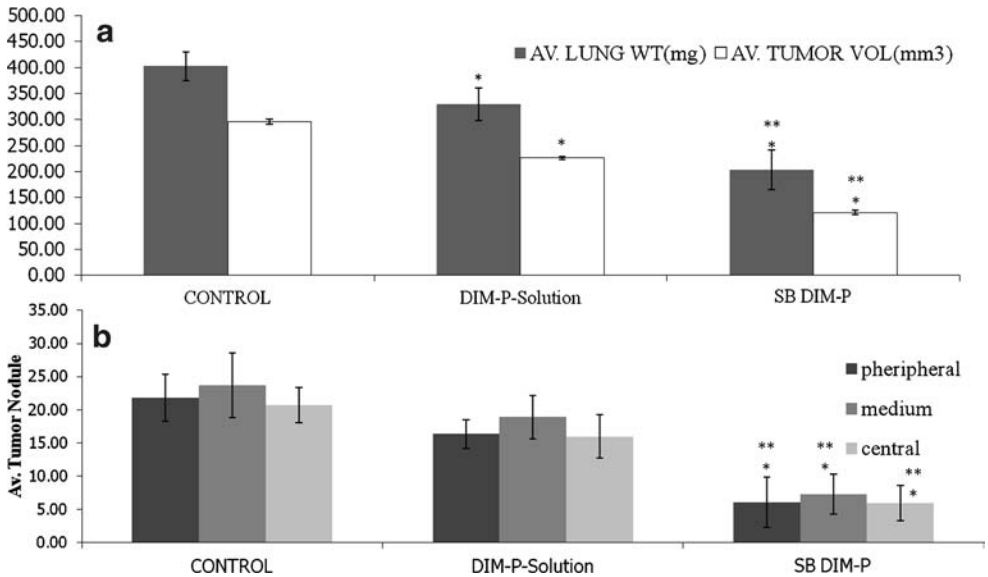

Fig. 4 Effects of DIM-P and SB DIM-P on metastatic H1650 lung tumor weight and tumor volume (**a**); and tumor nodules in central, mid and pheripheral regions of lungs (**b**). H1650 cells were injected into the nude mice via tail vein. Tumors were established for 10 days before therapy. Tumors from animals treated with DIM-P and SB DIM-P. Lung weights and tumor volumes were determined for measurement of therapeutic activity of the treatments. Tumor nodules of 2–10 mm^3 in volume were counted using harvested lungs for control and treated groups and the average number of tumor nodules were determined. One-way ANOVA followed by post Tukey test was used for statistical analysis. $P < 0.05$ (*Significantly different from untreated controls, **Significantly different from DIM-P solution). Data presented are means ± SD ($n = 12$).

DISCUSSION

In the current study, DIM-P was developed into a self-emulsified drug delivery system in solid form using a novel dual channel single spray drying technology (SB DIM-P) to overcome solubility and bioavailability problems (2). The formation of amorphous solid dispersions of lipophilic drugs by spray drying improves the dissolution profile, increases solubility and enhances the oral bioavailability of poorly soluble drugs. A specially designed spray gun simultaneously generates micro-particles along with a spray of enteric polymer which makes the micro-particles very uniformly coated. Conversion of liquid form of the self-emulsifying formulations into solid dosage forms by spray drying retains the advantage of self-emulsified systems to improve oral bioavailability and overcome the limitations of dosage form design and development.

Spray drying technology has been used by various investigators to enhance the bioavailability of poorly soluble drugs. For example, Hoffmeister *et al* used spray drying technology to develop redispersible spray-dried melatonin-loaded nanocapsules for controlled release delivery (26). Improved solubility, dissolution and bioavailability of pioglitazone by forming spray dried cyclodextrin inclusion complexes has also been reported (27). A fourfold higher lung deposition of budesonide micro particles using spray-drying compared to the conventional formulation was observed (28,29). Moreover, studies with delivery of other drugs including Candesartan, Cilexetil and lovastatin have also been improved using the spray drying approach (30,31).

For development of SEDDS formulations, the selection of suitable oil is crucial because it solubilizes the lipophilic drug and increases its transport via the intestinal lymphatic system, thus enhancing its absorption from GIT. Based on our solubility screening, since both sesame and corn oil had the maximum solubility for DIM-P, sesame oil was selected as oil phase because it contains higher amount of triglycerides with medium chain fatty acids; with lower interfacial tension provides better water solubility and partitioning as an emulsifier than triglycerides with long chain fatty acids (32). We also screened various non-ionic surfactants with HLB values >10 for their efficiency as emulsifier and Tween 20 (HLB 16) were selected as the surfactant for preparation of the binary mixture. The final composition of sesame oil and tween 20/ (1:1) resulted in formation of a stable self-emulsion on gentle agitation with water. In SEDDS, the visual estimation and rate of emulsification were means for the assessment of the efficiency of emulsification. The optimized formulation was emulsified within 55 s (rapidity of the formulation).

The SEDDS and SB DIM-P formulations were optimized using desirability functions such as droplet size and drug release, where theoretical and observed values were in close agreement. Higher values of correlation coefficient (R2) for the dependent variables indicated a good fit for SEDDS experimental model. The amount of drug/oil affects the particle size and the particle size was also influenced by the orifice of the needle and the viscosity of the solution. The SB DIM-P formulations were further optimized using desirability functions, where particle size was minimized and drug release was maximized, in order to have desired characteristics in the product. Airflow and feed concentration parameter of spray drying process had influence on each of the following property, such as: production yield, particle size and in vitro release profile. These properties can be altered to give desired values by adjusting airflow, feed rate and feed concentration of enteric coating solution. Validation in the laboratory demonstrated that the spray-drying process was reproducible ($0.075 < \text{S.D. repeatability} < 2.15$ and $0.085 < \text{S.D. reproducibility} < 1.98$). No significant variability in the spray-dried particle characteristics was observed between different batches suggesting that they are suitable for effective scale-up.

In-vivo pharmacokinetic analysis with the desired formulation in rats and dogs showed significantly ($p < 0.05$) higher absolute oral bioavailability of 56 and 26% respectively, compared to DIM-P solution. The increased AUC and C_{max} values with SB DIM-P formulation demonstrated the superior oral performance of our formulation compared to drug in free form. The relative bioavailability of the SB DIM-P was ~293% in dogs compared to ~302% in rats. These support the hypothesis that SB DIM-P is effective in improving the oral bioavailability of DIM-P. The plasma concentration of the DIM-P solution reached Cmax within 360 min after oral administration. Compared to DIM-P solution, the plasma concentration profile of the SB DIM-P was a little faster during the first 120 min, and was stabilized in sustained fashion over 720 min, followed by rapid decline in DIM-P plasma concentration, resulting in sustained release effect of SB DIM-P formulation. Also, bio-distribution of DIM-P following I.V. administration was found to be three compartment distribution in rats as seen previously (2). Since this is the first study of DIM-P in dogs, we do not have any previous information but we observed similar three compartment distribution. The data suggests that dogs are significantly different from rats for solution and SB DIM-P formulations for Cmax and AUC but are not significantly different from rats for both solution and SB DIM-P formulations for tmax. Several factors may have affected the pharmacokinetic profile between these species (e.g., gastric pH, surface area within the intestine, regional transit time, motility, interdigestive migrating myoelectric complex, composition and volume of GI fluids). DeSesso and Williams (33) have estimated surface area in the small intestine in dog is 168–210 m^2 compared to 24.75–35 m^2 in rat. The transit time in rat ranges from 0.5 to 1 h, whereas in the dogs, it varied from 2 to 4 h (33). The SEDDS provide better absorption in the intestine with lipophilic drugs, may be due to the microemulsions (droplet size in the range of 50 nm)

formation allows absorption via transcellular pathway and may protect the drug from enzyme degradation (34,35). Therefore, the higher bioavailability of DIM-P through SB DIM-P may be due to the enhanced lymphatic absorption (36,37). Also, a high ratio of emulsifier may provide increased permeability by disturbing the cell membrane (38).

Further, anticancer evaluation of SB DIM-P in A549 and H1650 lung tumor model in mice showed significant increase in anticancer activity than that of the DIM-P solution. This is expected because of the improved oral absorption and increased half-life of SB DIM-P. The improved oral bioavailability of DIM-P in SB DIM-P is further complemented by the increased apoptosis compared to DIM-P solution at the same dose of 20 mg/kg. DIM-P was first identified as a peroxisome proliferator activated receptor γ (PPARγ) and other C-DIMs containing p-hydroxyl or p-methoxyl substituted inactivate or activate the orphan receptor NURR1 (TR3). These compounds also induce apoptosis through receptor independent pathways and this includes activation of stress kinases such as JNK, induction of endoplasmic reticulum (ER) stress and disruption of mitochondria. In this study we have compared the relative in vivo anticancer activities of oral SB DIM-P vs oral DIM-P solution at the same dose of 20 mg/kg in an orthotopic lung tumor model. The dose of DIM-P used in this study was sufficient to inhibit lung tumor growth (based on previous study) and we compared the relative potencies of DIM-P vs SB DIM-P for several different responses. DIM-P and SB DIM-P did not induce any signs of toxicity. It was also evident that SB DIM-P was more potent than DIM-P with respect to inhibition of lung tumor weights (54 vs 26%) ($P<0.05$), lung tumor volumes (59 vs 29%), DNA fragmentation (apoptosis) in tumors (49 vs 22%) ($P<0.05$), decreased VEGF expression in tumors (58 vs 26%) ($P<0.01$) and induction of p-JNK (4.6 vs 1.8 fold) ($P<0.01$). These results demonstrate that SB DIM-P was highly effective for enhancing the anticancer potency of DIM-P and we are currently investigating the application of this delivery system for other C-DIMs that target oncogenic nuclear receptors such as NURR1 in lung and other tumor types.

CONCLUSION

The improved oral bioavailability and superior anticancer effect of DIM-P in lung cancer model through the self-emulsified and uniquely designed spray dried formulation, gives a novel opportunity and technology to deal with poorly water soluble and low oral bioavailable drugs. Based on superior pharmacokinetic and pharmacodynamic profile, self emulsified spray dried form of DIM-P can be a potential anticancer drug to treat/prevent different cancer types by oral administration. In addition, this unique dual channel mode spray drying system can be used for the simultaneous preparation of solid particles with enteric coating to protect from the gastric degradation. In conclusion, these approaches have very good industrial application to deal with poorly water soluble, gastric acid sensitive, first pass metabolizing drugs.

ACKNOWLEDGMENTS AND DISCLOSURES

The authors acknowledge the financial assistance provided by (DOH Grant ID#1KF07) James & Esther King Biomedical Research Program "Technology Transfer Feasibility (TTF) Grants". Dual Channel Spry Dryer and Modified Multilayered Microstructure for Oral delivery. U.S. Patent Filed in 2012 (US 13/368,993).

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