Determination of Minimum Effective Dose and Optimal Dosing Schedule for Liposomal Curcumin in a Xenograft Human Pancreatic Cancer Model

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Abstract. *Background: Curcumin is a food chemical present in tumeric (Curcuma longa) that has pharmacological activity to suppress carcinogenesis and inhibits multiple signaling pathways such as nuclear factor kappaB (NF-κB), cyclooxygenase-2 (Cox-2) and interleukin-8 (IL-8). Oral curcumin has poor oral bioavailability limiting its clinical activity; however, a patent pending liposomal formulation of curcumin was developed to improve drug delivery and has demonstrated activity in multiple cancers. This study was designed to determine the minimum effective dose (MED) as well as the optimal dosing schedule of liposomal curcumin in a xenograft mouse model of human pancreatic cancer. Materials and Methods: The MED determination and optimal schedule was evaluated in female athymic nude mice injected subcutaneously with MiaPaCa-2 cells. Dosing was initiated at an average tumor size of 5mm. For the MED, mice were treated with the following dose levels of liposomal curcumin: no treatment, liposome only, 1 mg/kg, 2 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg given by tail vein injection three times weekly for 28 days. For the optimum dosing schedule, three additional schedules were evaluated and compared to the control of three times weekly; daily (five days per week), every four days, and weekly for 28 days. All mice were weighed and tumor measurements taken three times weekly to evaluate toxicity and efficacy. Results: The 20 mg/kg dose had the greatest decrease in tumor growth at 52% decrease in tumor growth when compared to no treatment control mice. MED was determined to be 20 mg/kg and was used for the optimal dosing schedule determination. Daily dosing and three times per week dosing had greater inhibition of tumor growth with no discernable difference than once weekly or every 4 day dosing. No toxicity was observed at any dose or schedule. Conclusion: The MED for liposomal curcumin is 20 mg/kg given once daily three times per week to achieve optimal tumor growth inhibition. This was dose recommended for additional preclinical studies to define safety and tolerability of liposomal curcumin in rat and dog models.*

Pancreatic cancer is the fourth leading cause of death in the United States. In 2008 it is estimated that 37,680 will be diagnosed with pancreatic cancer and 34,290 will die from this cancer. Pancreatic cancer has a very poor prognosis and mortality closely follows the incidence of disease with a near 99% mortality rate at 5 years (1, 2). Currently the only FDA approved chemotherapeutic agent indicated for treatment of pancreatic cancer is gemcitabine with minimal improvement on disease survival at best. New therapies that specifically target the molecular pathways of pancreatic cancer are desperately needed to improve survival and increase curative potential in this disease.

Curcumin (diferuloylmethane) is a food chemical present in tumeric (*Curcuma longa*) has confirmed pharmacological safety as demonstrated by its long history of consumption as dietary spice for centuries (3). It has exhibited antioxidant, anti-inflammatory, antimicrobial, and anticancer activity (3-11). Focusing on the oncology setting, *in vitro* studies have reported curcumin has potent antiproliferative and proapopototic activity exerted through multiple molecular pathways (12-15). Curcumin is a known potent inhibitor of NF-κB and results in the down regulation of COX-2 and IL-8 which are often expressed at increased levels in pancreatic cancer (15). Numerous human clinical studies have proven curcumin is extremely safe with doses up to 12 grams per day being well tolerated (16). However, this potential therapy is complicated

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Key Words: Curcumin, liposomal, dose finding, pancreatic, cancer.

Table I. *Liposomal curcumin dose levels for the minimum effective dose (MED) finding study.*

Control Groups	Liposomal Curcumin Treatment Groups (all administered once daily three times per week)
No treatment	1 mg/kg
Liposome only	2 mg/kg
	5 mg/kg
	10 mg/kg
	20 mg/kg
	40 mg/kg

Table II. *Dosing regimens evaluated to determine the optimal dose regimen for liposomal curcumin.*

Control dose regimen	Liposomal curcumin 20 mg/kg modified dose regimens
Liposomal curcumin	Once per week
20 mg/kg given once daily	Once daily every four days
three times per week	Once daily times five days per week

by very poor bioavailability and lack of solubility which limits potential intravenous (IV) formulations. For example in a pharmacokinetic study by Shoba and colleagues, human plasma concentrations achieved were undetectable with maximum concentrations less than 5 ng/mL at one hour (17). Hence, a major focus of research in this area has been to develop a formulation to improve drug delivery either by oral or IV route.

Kurzrock and colleagues developed a novel liposomal formulation of curcumin for intravenous administration (15). The lipid to curcumin is in 10:1 ratio and is prepared 5 mg/mL solution. In the preliminary mouse safety and efficacy studies, the selected dose of 40 mg/kg three times a week was based upon the maximum volume able to be administered with no toxicity in animal models which demonstrated significant activity in pancreatic tumor model (15). Alternative dosing regimens had not been reported. The objective of this current study was to define the minimum effective dose (MED) and optimal dosing schedule for liposomal curcumin in preparation for human clinical studies.

Materials and Methods

Cell culture. The human pancreatic cell line MIA PaCa-2 was purchased from American Type Culture Collection (Manassas, VA, USA) and cultured using DMEM with high glucose , pyruvate, glutamine (The University of Texas M.D. Anderson, Media Core Facility) and supplemented with 10% fetal bovine serum (FBS) purchased from Sigma-Aldrich Company (St. Louis, MO, USA).

Liposomal curcumin. Liposomal curcumin was prepared by the method described by Li *et al*. (15) (patent pending 60/452,630) using the following material: 1,2 Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] [sodium salt] (DMPG) dry powders were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Curcumin (diferuloylmethane) ((1E, 6E)-1.7-Bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) was provided by Sami Labs Limited (Karnataka, India).

Analytical assay. The concentration of curcumin in each batch of prepared liposomal curcumin was verified by high-performance liquid chromatography adapted from the method of Heath and colleagues (18). Briefly, curcumin was isolated from spiked, salvaged rat plasma by liquid/liquid extraction with acetonitrile: methanol: acetic acid (63:35.5:1.5). Liquid chromatographic separation was achieved by isocratic elution on a Waters Nova-Pak C18, 3.9×150 mm, 4 μm particle size packing analytical column. The mobile phase consisted of a composition of acetonitrile: methanol: diH_2O: acetic acid (41:23:36:1), and measured to have a pH of 3.3. The flow rate was 0.5 mL/min. The run time was set for 10 minutes for each sample. The curcumin peak was positively identified from other peaks using PDA absorbance at a wavelength of 430nm with retention time for curcumin was 5.6±0.2 minutes. Peak area amounts were proportional to curcumin over the plasma concentration range from 100 ng/mL to 4,000 ng/mL.

Animal model. Female athymic nu/nu mice (4-6 weeks old) obtained from Charles Rivers Laboratories (Wilmington, MA, USA) were maintained three per cage in micro isolator units. Mice were fed commercial autoclavable diet and water. Mice were injected with 5×10^6 MIA PaCa-2 cells in 100 μL were injected subcutaneously into the left flank of the mice. Once tumor masses became established with an average tumor size of 5mm, mice were randomized to treatment groups and liposomal curcumin or liposome only were administered as a maximum volume of 0.2 mL *via* tail vein injection. Three mice were in each treatment arm with a total of 30 mice to complete both studies.

Minimum effective dose (MED) study. Eighteen mice were randomized to be treated with the following dose levels of liposomal curcumin with three mice per dose level: no treatment, liposome only, 1 mg/kg, 2 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg given by tail vein injection. The initial dose schedule of three times weekly for 28 days (Table I) was selected based upon preliminary mouse safety and efficacy studies (15). All mice were weighed and tumor measurements taken three times weekly to evaluate toxicity and efficacy. Upon completion of the MED study, all mice were sacrificed, the subcutaneous tumors were harvested, weighed and saved for potential future molecular confirmation studies.

Optimal dosing schedule study. After the MED was determined twelve mice randomized to receive the MED with each of the following dosing schedules over 28 days with three mice per treatment group: once weekly, once every four days, or once daily times five days per week. These modified dosing schedules were compared to the control schedule of three times per week dosing (Table II). All mice were weighed and tumor measurements taken three times weekly to evaluate toxicity and efficacy. Upon completion of the dosing schedule study, all mice were sacrificed, the subcutaneous tumors harvested, weighed and saved for potential future molecular confirmation studies.

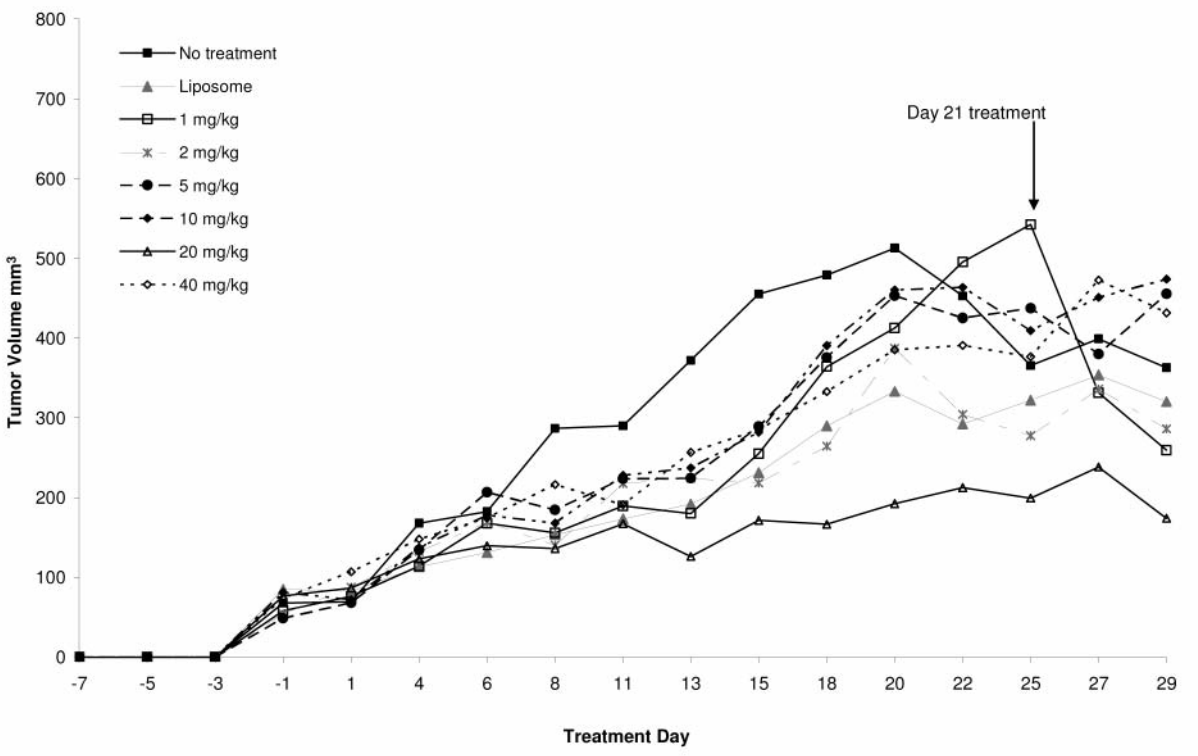

Figure 1. *Mean tumor volume over duration of the liposomal curcumin minimum effective dose (MED) finding study. The curves of the eight groups illustrating the mean tumor volume of mice non-treated, diluent alone, and different treatment arms of liposomal curcumin (1 mg/kg to 40 mg/kg) for the MiaPaCa2 pancreatic cancer xenograft model. The 20 mg/kg liposomal curcumin arm demonstrated a consistent greater inhibition of tumor growth compared to all other treatment arms.*

Results

Minimum effective dose finding results. The MED for liposomal curcumin in this mouse xenograft model of human pancreatic cancer was determined to be 20 mg/kg. After 21 days of treatment, a maximum of 62.5% inhibition of tumor growth was achieved with the 20 mg/kg dose level compared to the no treatment control group (Figure 1). At time of sacrifce, the tumor mean weight in the 20 mg/kg was 57.8% less compared to mean tumor weight in the no treatment control group (Figure 2). No toxicity was observed with liposomal curcumin at any dose with total body weights remaining stable throughout the experiment.

Dosing schedule pptimization results. There was less than 6% difference in mean tumor volume between the three times per week and once daily times five days per week dosing schedule (Figure 3). The three times per week demonstrated a 25.6% and 43.2% improvement in tumor growth inhibition compared to the once every four days and once per week dosing schedules (Figure 3). No toxicity was seen with liposomal curcumin for any schedule with mouse weights remaining stable throughout the experiment.

Discussion

The focus on the evaluation of nutritional supplements and herbal products as potential therapeutic agents for the treatment of cancer has become increasingly more common into Western world oncology clinical practice. However, before we can define the role of each nutritional supplement and herbal product in conventional treatment of cancer, more information is needed about the complex pharmacology of these agents in appropriate pre-clinical studies to confirm safety and limit potential drug-drug interactions as we proceed to design and initiate prospective clinical studies to establish the role of herbal and nutritional therapies into current clinical practice.

Curcumin (diferuloylmethane) is a food chemical present in tumeric (*Curcuma longa*) that has confirmed pharmacological activity such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activity (3-11). Recent *in vitro* studies have established its potent antiproliferative and proapopototic activity exerted through multiple molecular pathways such as inhibition of NF-κB (12-15). Numerous human clinical studies have demonstrated oral doses of curcumin are safe with doses up to 12 grams per day

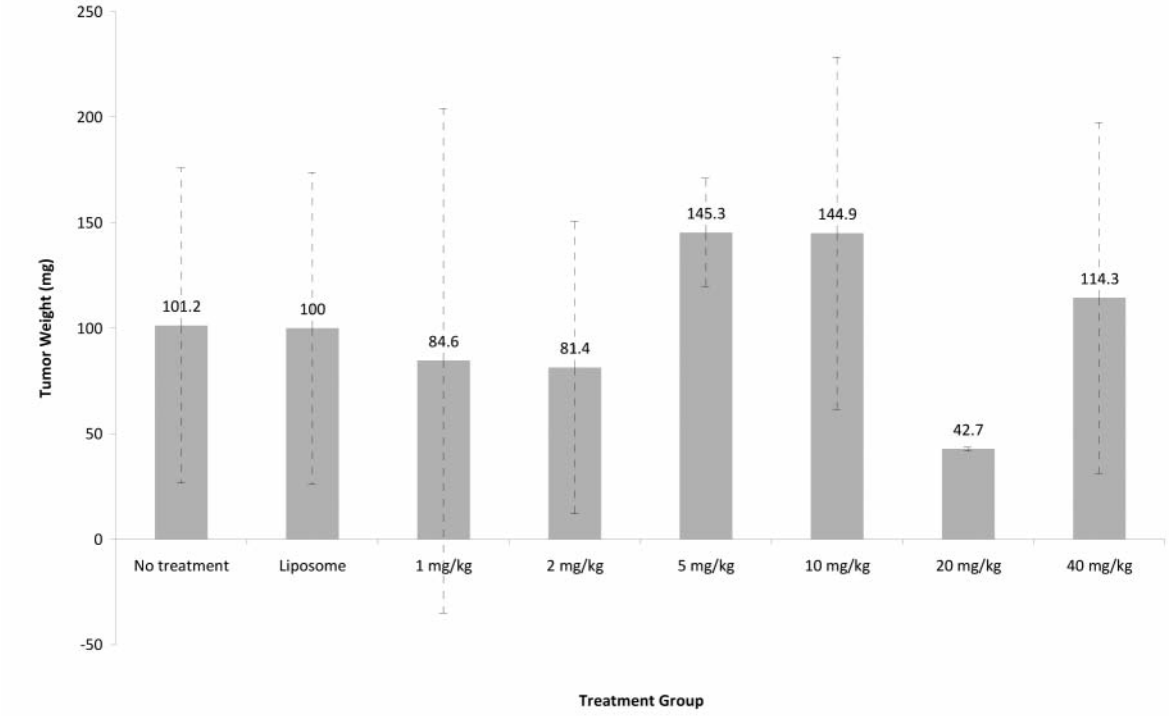

Figure 2. *Comparison of the final mean tumor weight at each dose liposomal curcumin dose level. The bars represent the final mean tumor weight for each of the eight groups of mice including non-treated, diluent alone, and different treatment arms of liposomal curcumin (1 mg/kg to 40 mg/kg) for the MiaPaCa2 pancreatic cancer xenograft model. Dash lines represent standard deviation for each treatment arm. The liposomal curcumin 20 mg/kg arm achieved greatest inhibition of tumor growth with the least variability compared to all other treatment arms.*

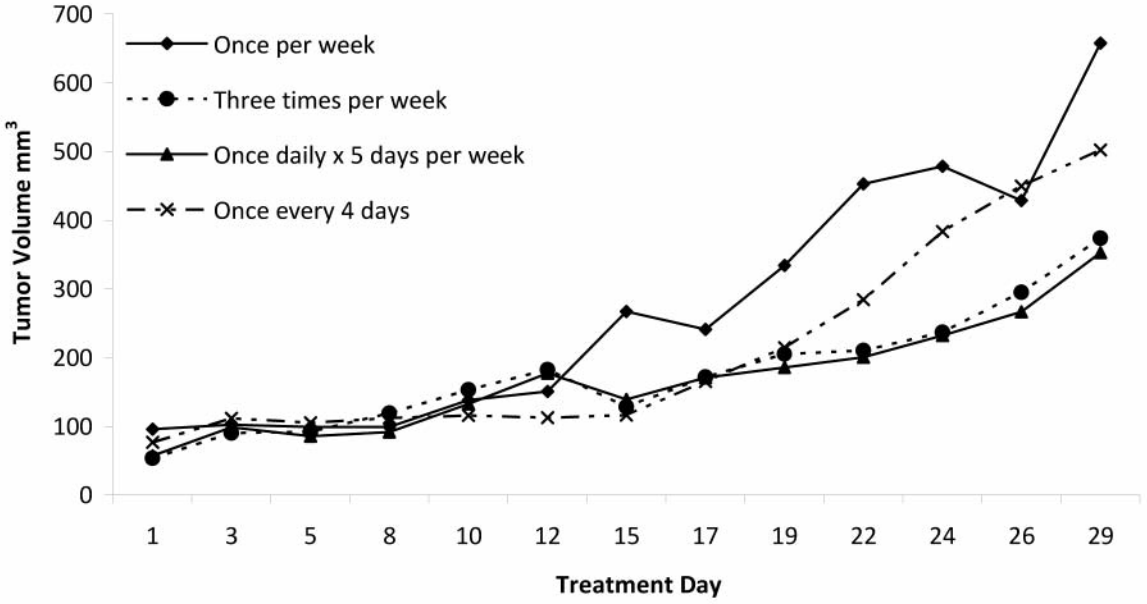

Figure 3. *Mean tumor volume over duration of the study to determine the optimal dose regimen for liposomal curcumin. The four curves illustrate the mean tumor volume of liposomal curcumin 20 mg/kg given either once per week, once daily three times a week; once daily times five days per week or once daily every four days in the MiaPaCa2 pancreatic cancer xenograft model. The liposomal curcumin 20 mg/kg once daily three times a week versus once daily five times a week were similar and demonstrated better inhibition of tumor growth compared to the other treatment arms.*

but with fairly low or undetectable plasma concentrations being achieved (16, 17). Research in this area has been focusing on the development of alternative formulations to improve drug delivery either by oral or IV route.

This study focused on dose finding studies for a novel liposomal formulation of curcumin for intravenous administration. The MED for liposomal curcumin in this mouse xenograft model of human pancreatic cancer was determined to be 20 mg/kg, which will allow for a decrease in the volume necessary to administer this dose as development moves forward. After the MED was defined, the optimal dosing regimen was evaluated to determine not only most effective but also what would be feasible in clinical setting both in respect to time and cost. Although the once daily dose five times per week versus the once daily three times per week dosing of liposomal curcumin were equivalent activity, once daily three times weekly dosing was selected as the more clinically feasible regimen. At all dose levels and regimens the liposomal curcumin does not have any apparent dose limiting toxicity and was well tolerated in this mouse model. This regimen will be evaluated in additional animal models prior to initiating confirmatory studies in humans.

Acknowledgements

This research was funded by unrestricted research grant from Sign Path Pharma, Inc.

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Received March 6, 2009
Accepted April 29, 2009