Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer

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Abstract

The role of curcumin (diferuloylmethane), a proapoptotic compound, for the treatment of cancer has been an area of growing interest. Curcumin in its free form is poorly absorbed in the gastrointestinal tract and therefore may be limited in its clinical efficacy. Liposome encapsulation of this compound would allow systemic administration. The current study evaluated the preclinical antitumor activity of liposomal curcumin in colorectal cancer. We also compared the efficacy of liposomal curcumin with oxaliplatin, a standard chemotherapy for this malignancy. *In vitro* treatment with liposomal curcumin induced a dose-dependent growth inhibition [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt] and apoptosis [poly(ADP-ribose) polymerase] in the two human colorectal cancer cell lines tested (LoVo and Colo205 cells). There was also synergism between liposomal curcumin and oxaliplatin at a ratio of 4:1 in LoVo cells *in vitro*. *In vivo*, significant tumor growth inhibition was observed in Colo205 and LoVo xenografts, and the growth inhibition by liposomal curcumin was greater than that for oxaliplatin ($P < 0.05$) in Colo205 cells. Tumors from animals treated with liposomal curcumin showed an antiangiogenic effect, including attenuation of CD31 (an endothelial marker), vascular endothelial growth factor, and interleukin-8 expression by immunohistochemistry. This study establishes the comparable or greater growth-inhibitory and apoptotic effects of liposomal curcumin with oxaliplatin both *in vitro* and *in vivo* in colorectal cancer. We are currently developing liposomal curcumin for introduction into the clinical setting. [Mol Cancer Ther 2007;6(4):1276–82]

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Introduction

Colorectal carcinoma represents one of the most frequent causes of cancer death in the Western population. Treatment of colorectal cancer generally consists of a combination of three classic strategies of oncology: surgery, radiation, and chemotherapy. There are now several therapeutic drugs that have shown efficiency in colorectal cancer, although metastatic disease remains mostly incurable (1). One of the more potent drugs used in this disease is oxaliplatin, a third-generation platinum compound that forms intrastrand links between two adjacent quinine residues or a guanine and adenine, hence disrupting DNA replication and transcription (2). Oxaliplatin has significant antitumor activity in colorectal cancer and has been approved for this indication (3).

Curcumin (diferuloylmethane) is a phytochemical that has potent antiproliferative effects against a variety of tumors *in vitro*. It exerts its effects via diverse biological properties, including, but not limited to, suppression of nuclear factor-κB and inhibition of angiogenesis (4–10). It also enhances the antitumor effects of several classic chemotherapeutic drugs, such as doxorubicin, *cis*-platinum, and paclitaxel (11–13). Curcumin, however, is limited in its clinical utility because of its poor bioavailability. To circumvent this limitation, we have recently developed a liposomal formulation of curcumin that can be administered i.v. Previously, we have shown that this formulation has antiproliferative and proapoptotic effects equal to or greater than that of free curcumin against human pancreatic cancer cell lines *in vitro* (8).

A mainstay of the strategy for cancer treatment involves using combination therapy often with drugs that have differing mechanisms or nonoverlapping toxicities. Because curcumin is nontoxic, and may potentiate certain chemotherapeutic agents, it has potential for use in combination regimens (11–14).

In the current study, we show that liposomal curcumin has antitumor effects against colorectal cancer both *in vitro* and *in vivo*, that its effects are equal to or greater than those of oxaliplatin, and that it acts as an antiangiogenic agent. In addition, when combined at a 4:1 ratio with oxaliplatin, synergistic effects are obtained. These results suggest that liposomal curcumin alone, or in combination with oxaliplatin, may be a useful strategy for the therapy of colorectal cancer.

Materials and Methods

Cell Lines

The human colorectal cancer cell lines Colo205 and LoVo cells were purchased from the American Type Culture Collection (Manassas, VA). Colo205 cells were grown in

RPMI 1640 and LoVo cells were grown in DMEM-F12, both supplemented with 10% FCS. All media were purchased from Invitrogen (Carlsbad, CA).

Lipids, Chemicals, and Curcumin

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy-(polyethylene glycol)-2000], and 1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) were obtained as a dry powder from Avanti Polar Lipids (Alabaster, AL). SyntheChol (synthetic cholesterol), curcumin, acetone, and *tert*-butanol were obtained from Sigma Chemical Co. (St. Louis, MO). Oxaliplatin (5 mg/mL; Sanofi-Synthelabo, Bridgewater, NJ) was purchased from Ben Venue Laboratories (Bedford, OH). For *in vivo* experiments, stock solution of oxaliplatin was diluted 5 to 10 times in 5% glucose solution for injection as recommended by the supplier.

Liposomal Preparation

The lipids used were 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine/1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) or a pegylated version of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine/cholesterol/1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy-(polyethylene glycol)-2000]. Different total lipid to curcumin ratios (w/w) ranging from 10:1 to 4:1 were tested before settling on a fixed ratio of 10:1 based on tests to determine optimal encapsulation of curcumin by liposomes. The lipids and curcumin were dissolved in *tert*-butanol and filtered through a 0.22-μm pore size filter for sterilization. The vials containing lipids and curcumin (10:1 ratio) solution were frozen in a dry ice-acetone bath and lyophilized for 24 h to remove the *tert*-butanol. The vials were stored at −20°C and warmed up to room temperature just before use.

Cell Proliferation

The effects of liposomal curcumin and oxaliplatin on colorectal cancer cell line proliferation were determined with the tetrazolium-based [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt] assay referred to as CellTiter 96 (Promega, Madison, WI). The assay was done in triplicate. LoVo and Colo205 cells were plated in 96-well plates at 5×10^3 per well overnight. Cells were treated in triplicate with various concentrations of liposomal curcumin, empty liposomes, and oxaliplatin or combinations thereof were incubated for 72 h. Medium was aspirated from the wells; the cells were rinsed with PBS twice. Fresh medium (200 μL) was added to each well followed by addition of 20 μL dye solution and incubated at 37°C for 4 h. Absorbance was measured at 490-nm wavelengths using an ELISA plate reader (Molecular Devices, Sunnyvale, CA).

Immunoblotting

Immunoblotting using standard procedures was done to assess poly(ADP-ribose) polymerase (PARP) cleavage (reflecting apoptosis). A protein assay to measure protein content was done by using the bicinchoninic acid protein assay kit (Pierce Endogen, Rockford, IL). The samples were fractionated by a 6% to 10% SDS-PAGE and electroblotted on nitrocellulose membrane. The signal was detected by secondary antibody [horseradish peroxidase–conjugated anti-mouse Ig or anti-rabbit Ig, as appropriate (1:5,000); Amersham Pharmacia Biotech, Piscataway, NJ] and the enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech) and then autoradiographed.

Animals

Colorectal cancer s.c. xenografts were created in female athymic *nu/nu* mice (3–5 weeks; Harlan Sprague Dawley, Inc., Indianapolis, IN). Mice were maintained at five per cage in microisolator units. Animals were given a commercial diet and water. Mice were quarantined for at least 1 or 2 weeks before experiment manipulation. All of the animal experiments were done at The University of Texas M. D. Anderson Cancer Center (Houston, TX) under protocol 10-02-13231 approved by the Institutional Animal Care and Use Committee of The University of Texas M. D. Anderson Cancer Center.

In vivo Antitumor Activity of Liposomal Curcumin and Oxaliplatin in Human Colorectal Tumor Xenografts in Nude Mice Models

Colo205 and LoVo (1×10^7) cells collected in 100 μL culture medium in log-phase growth were injected s.c. into one side of the flank of female nude mice (3–5 weeks of age; Harlan Sprague Dawley). Once tumor masses became established at 3 days (tumor volume, ~20–30 mm^3 for LoVo and ~100 mm^3 for Colo205), animals were randomized to receive i.v. tail vein (40 mg/kg body weight) injections thrice weekly (this is the maximum volume that could be injected). Injections consisted of empty liposomes, liposomal curcumin, and oxaliplatin (i.p. 5 mg/kg), alone or in combination. Tumor size and body weight were measured with a caliper thrice weekly. The tumor volume was calculated using the following formula: volume = (length × width^2) / 2, where width was the shortest measurement in millimeter.

Histologic Sections

Formalin-fixed tissue was paraffin embedded, sectioned (3–5 μm), and stained with H&E. Section was evaluated for tumor cell cytology, necrosis, and associated inflammatory cellular response.

Immunohistochemistry for Angiogenesis

To determine angiogenic effects, we assayed for CD31, basic fibroblast growth factor, vascular endothelial growth factor (VEGF), and interleukin-8 expression in xenograft tissue before and after treatment. Immunohistochemical studies were done by using formalin-fixed, paraffin-embedded sections (5 μm), heat-induced antigen retrieval (Dako Corp., Carpinteria, CA), and 1:50 to 1:200 monoclonal concentrations of antibody. The frozen sections were cut from snap-frozen tissue and embedded in OCT compound (Miles, Elkhart, IN). The detection system was the LSAB2 detection kit (Dako). The secondary antibody is a biotinylated antibody (Dako) and forms a complex with peroxidase-labeled streptavidin. Counterstaining was done by using Gill's hematoxylin (Sigma Chemical). Negative controls were done by replacing the primary antibody with

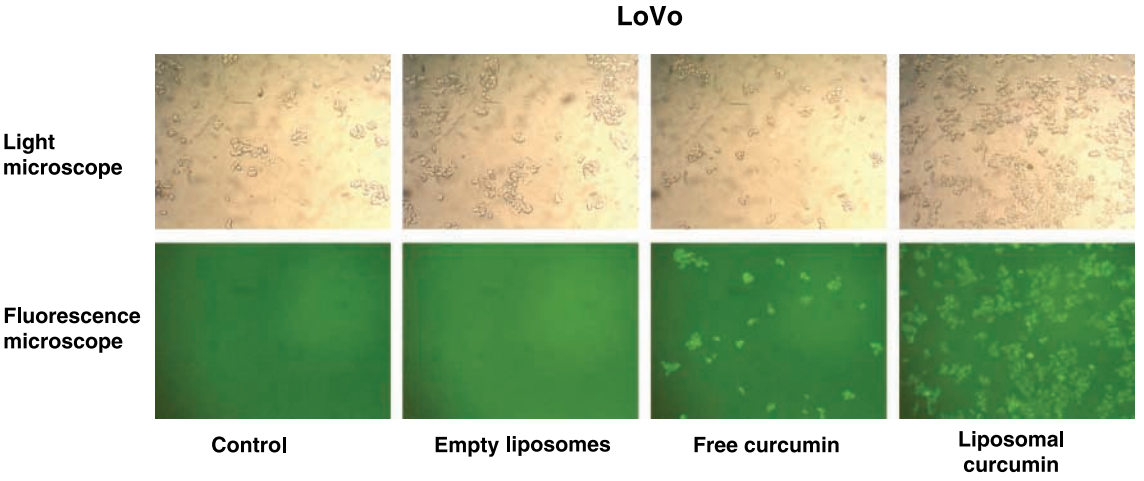

Figure 1. Internalization of liposomal curcumin versus free curcumin. Light and fluorescence micrographs show representative internalization at 2 h after treatment with 10 μmol/L concentrations of pegylated liposomal curcumin versus free curcumin. Internalization is equal for both.

anti-IgG1 (the same isotype with the primary antibody; Oncogene, Boston, MA).

Antibodies

Antibodies used for immunoblotting included monoclonal anti-tubulin antibody (Sigma Chemical), anti-PARP rabbit polyclonal (Cell Signaling, Beverly, MA), anti-VEGF monoclonal antibody (Oncogene), rat anti-mouse CD31 (BD PharMingen, San Diego, CA), and polyclonal anti-interleukin-8 antibody (Biosource, Camarillo, CA).

Median Effect Analysis

Median effect analysis (CalcuSyn version 2, Biosoft, Ferguson, MO) was used to determine interactions between liposomal curcumin and oxaliplatin. The CalcuSyn program provides a measure of the combined drug interaction by the generation of a combination index (CI) value. Dose-response interaction (antagonism, additive, or synergism) was expressed as a nonexclusive case. Combination indices were calculated according to Chou and Talalay (14). CI < 1 indicates synergism, CI > 1 indicates antagonism, and CI = 1 indicates that the two drugs are additive.

Results

Liposomal Curcumin Internalizes in Cancer Cells as Easily as Free Curcumin

Liposomal curcumin was expected to internalize into cells as easily as free curcumin (Fig. 1). Our experiments using light and fluorescence microscopy showed nearly complete internalization of pegylated liposomal curcumin into the colorectal cells after 2 h of treatment with a 10 μmol/L concentration. Internalization was comparable with that of free curcumin (Fig. 1).

In vitro Growth Inhibitory Effect of Liposomal Curcumin Is Greater Than or Equal to That of Oxaliplatin

After 72 h of incubation, the IC_{50} of pegylated liposomal curcumin was less than that of oxaliplatin for LoVo cells as determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt assay (IC_{50} for liposomal curcumin = 7.5 μmol/L; IC_{50} of oxaliplatin was >40 μmol/L; Fig. 2). For Colo205 cells, the IC_{50} of oxaliplatin was equivalent to that of pegylated liposomal curcumin. Pegylated liposomal curcumin had antiproliferative effects equivalent to those of free curcumin (Fig. 2).

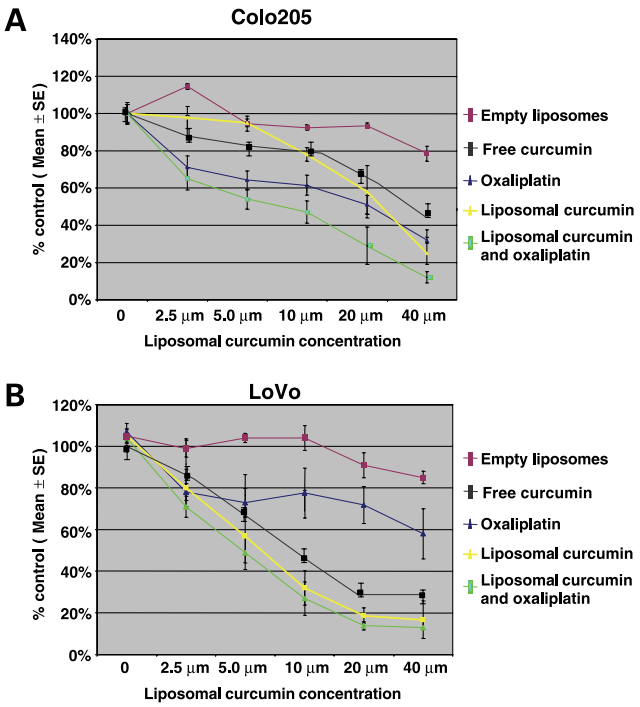

Figure 2. Inhibitory effect of liposomal curcumin and oxaliplatin on cell proliferation in colorectal cancer cells (**A**, Colo205 cells; **B**, LoVo cells). *Points,* mean of three independent experiments after 72 h of exposure to the above compounds; *bars,* SE. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt assay was used. Pegylated liposomal curcumin has growth-suppressive effects comparable with those of oxaliplatin in Colo205 cells. There was no significant difference between the effect of free curcumin and that of pegylated liposomal curcumin.

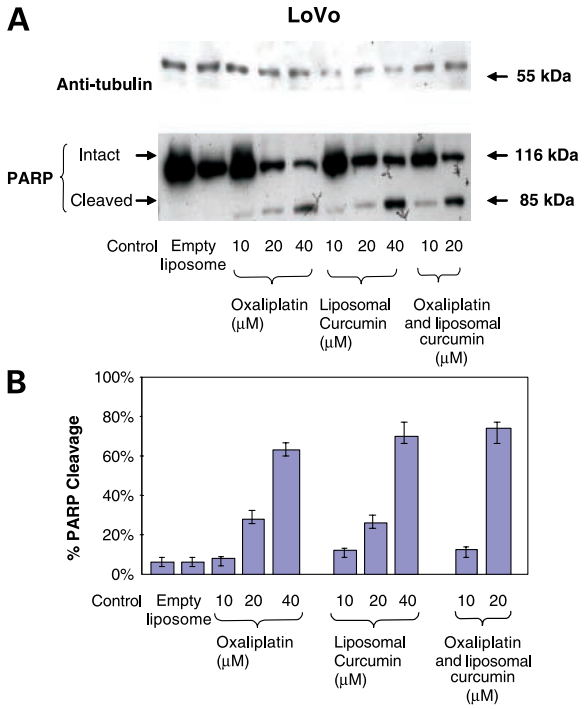

Figure 3. PARP cleavage determined by Western blot analysis in colon cancer cell lines. Colon cancer LoVo cells were treated with pegylated liposomal curcumin, oxaliplatin, and the combination of these two drugs for 72 h. PARP cleavage was evaluated at the end of the exposure. Cells were lysed and analyzed by SDS-PAGE and immunoblotted with anti-PARP antibody. **A,** Western blot. **B,** densitometry analysis results of three experiments. *Columns,* median; *bars,* SE. *Arrows,* position of both 116 and 85 kDa. There is a dose-dependent PARP cleavage after exposure to oxaliplatin, liposomal curcumin, or the combination of liposomal curcumin and oxaliplatin (for the combination of liposomal curcumin and oxaliplatin, 5 or 10 μmol/L of each compound were used to total 10 or 20 μmol/L, respectively, of the combination). In combination, liposomal curcumin (10 μmol/L) combined with oxaliplatin (10 μmol/L) was significantly more proapoptotic ($P < 0.05$) than either drug alone at 20 μmol/L (**B**).

PARP Cleavage after Liposomal Curcumin and Oxaliplatin Exposure *In vitro*

PARP is an enzyme involved in DNA damage and repair mechanisms, and cleavage reflects apoptosis. Figure 3 shows PARP cleavage as determined by Western blot analysis after 72 h of exposure of LoVo cells to pegylated liposomal curcumin, oxaliplatin, and the combination of these two drugs. Pegylated liposomal curcumin and oxaliplatin both induced PARP cleavage, but the combination of liposomal curcumin and oxaliplatin produced an increase in PARP cleavage in LoVo cells at 20 μmol/L (10 μmol/L of liposomal curcumin and 10 μmol/L of oxaliplatin) compared with that of 20 μmol/L of either drug alone (Fig. 3B).

Liposomal Curcumin Suppresses Colo205 and LoVo Tumor Growth in a Mouse Xenograft Model

Liposomal curcumin suppressed the growth of Colo205 and LoVo tumors in a murine xenograft s.c. model when compared with empty liposomes or saline (Fig. 4). Animals received 40 mg/kg of liposomal curcumin i.v. thrice weekly. The suppressive effects of liposomal curcumin were equal to or greater than those of oxaliplatin. No overt side effects were observed (but whether this molecule has toxicity in animals awaits formal toxicology studies). We also saw no significant changes in weight in liposomal curcumin-treated animals bearing xenografts compared with unimplanted animals treated with saline or empty liposomes.

Liposomal Curcumin Induces an Antiangiogenic Effect in Colo205 and LoVo Tumor Xenografts

It has been well documented that tumor growth and metastases require new vasculature. To determine whether the reduced growth of tumors after liposomal curcumin treatment of colorectal cancer xenografts was associated with an antiangiogenic effect, we examined the formation of neovasculature in Colo205 and LoVo tumor xenografts. Figure 5 shows that the number of cells stained with antibody against an endothelial cell marker (anti-CD31) was reduced in the treatment group tumor compared with the group treated with empty liposomes or saline. We also examined other critical angiogenic factors, such as VEGF, interleukin-8, and basic fibroblast growth factor, and found that levels were significantly reduced in the liposomal curcumin-treated group compared with the

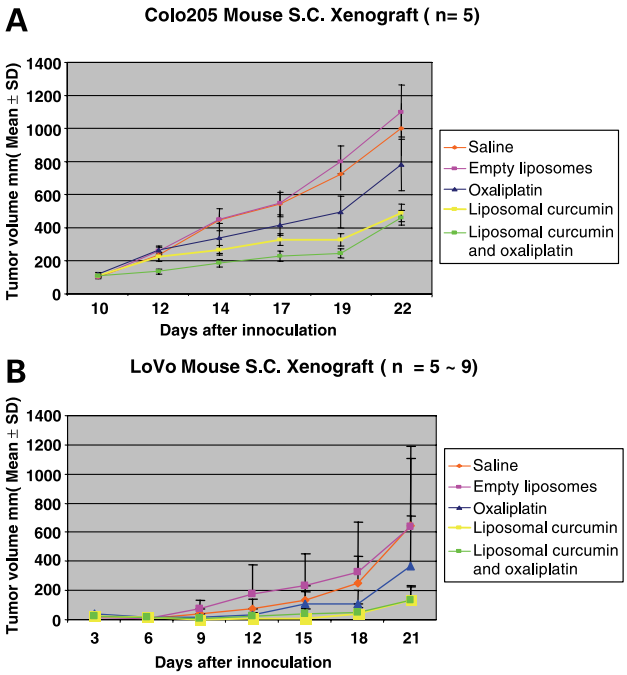

Figure 4. Effect of liposomal curcumin on colorectal tumor growth *in vivo*. Growth inhibition of human Colo205 (**A**) or LoVo (**B**) colorectal carcinoma xenografts in female nude mice after treatment with liposomal curcumin (40 mg/kg) i.v. and oxaliplatin (5 mg/kg) i.p., alone or in combination thrice weekly. Five mice were treated in each group. *Points,* mean of tumor volume; *bars,* SE. There was a significant growth inhibition after treatment with liposome curcumin alone and for combined liposomal curcumin and oxaliplatin compared with control (empty liposomes; $P < 0.05$) for tumor size on day 22.

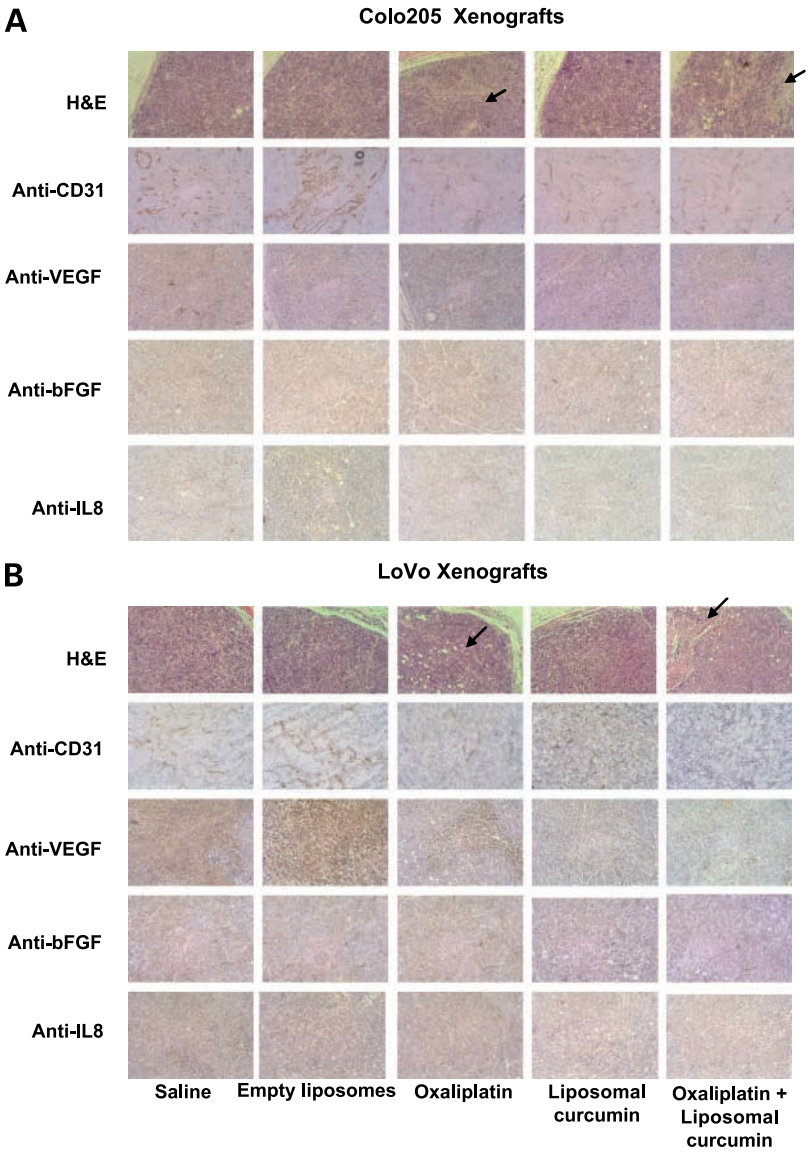

Figure 5. Effect of liposomal curcumin and oxaliplatin on the pathology and antiangiogenic expression of colorectal carcinoma. H&E-stained sections and immunohistochemical staining of tumor sections from Colo205 (**A**) and LoVo (**B**) xenografts. Tissue sections were analyzed by H&E, anti-CD31, anti-interleukin-8 (*Anti-IL8*), anti-VEGF, and anti-basic fibroblast growth factor (*Anti-bFGF*), counterstained with hematoxylin, and then photographed at ×100. *Arrow,* necrosis was observed in the oxaliplatin and oxaliplatin plus liposomal curcumin treatment group of both Colo205 and LoVo xenografts. Expression levels of all stains, except for basic fibroblast growth factor, were lower after therapy in LoVo cells, indicating antiangiogenic effects. In Colo205 xenografts, the effect was less pronounced with CD31 but not with other angiogenic markers after treatment.

controls (basic fibroblast growth factor levels were, however, unchanged in Colo205 cells). These results showed a potent antiangiogenic effect for liposomal curcumin.

Effect of Combinations of Liposomal Curcumin and Oxaliplatin

Combination therapy has been a cornerstone of treating cancer. We therefore examined the effect of combination of liposomal curcumin and oxaliplatin *in vitro* and *in vivo*. *In vitro* combinations of equimolar concentrations of oxaliplatin and liposomal curcumin resulted in no significant enhanced growth-inhibitory effect compared with that of either drug by itself (Fig. 2). However, Fig. 6 shows that combinations of liposomal curcumin and oxaliplatin at a 4:1 molar ratio resulted in synergy (median effect analysis). There was no synergy for the two drugs *in vivo* (Fig. 4).

Discussion

Plant-derived compounds have historically been some of the most commonly used and effective medicines. Several contemporary, evidence-based therapies, including chemotherapeutic drugs, such as paclitaxel and *Vinca* alkaloids, are plant derived. Many plant-derived compounds have the advantage of having a favorable therapeutic-to-toxicity index.

Curcumin (diferuloylmethane) is a component of the root of the plant *Curcuma longa*. Centuries of culinary use in the Far East suggest that this phytochemical is safe to ingest. In recent years, there has been a growing interest in the antitumor activity of curcumin. Most of the studies used free curcumin, which is poorly absorbed in the gastrointestinal tract and is therefore limited in its clinical efficacy (14, 15). The highly hydrophobic nature of curcumin makes i.v. dosing impossible. Liposome encapsulation of curcumin

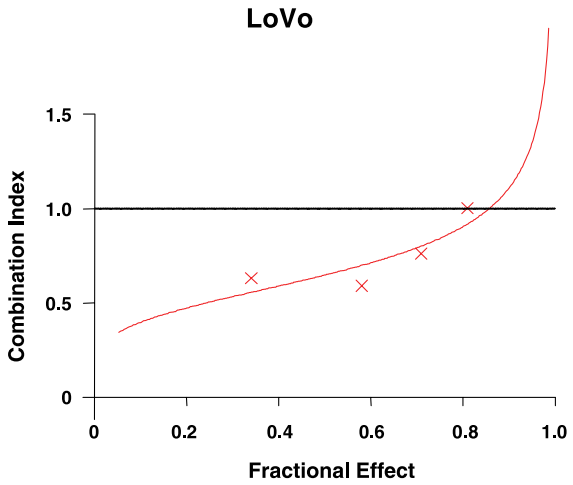

Figure 6. Median effect analysis of the combination of oxaliplatin and liposomal curcumin on LoVo cell lines *in vitro*. Cells were incubated with pegylated liposomal curcumin and oxaliplatin at micromolar ratios of 4:1 for 72 h followed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The fractional effects on cell death are calculated by following formula: (100 − alive cells) / 100. The CalcuSyn program was then used to calculate the two-drug interaction. CIs are presented as a function of fraction of cells affected by the cytotoxic effect. CI > 1.0 indicates antagonism, CI = 1.0 indicates additive effects, and CI < 1.0 indicates synergy. As shown, for our experiments, the 4:1 ratio of liposomal curcumin to oxaliplatin was synergistic.

makes this agent amenable to i.v. dosing without attenuation of its antitumor properties (8).

The current study evaluated the preclinical antitumor activity of liposomal curcumin in colorectal cancer. We also compared the efficacy of liposomal curcumin with oxaliplatin, a classic chemotherapy that is approved by the Federal Drug Administration for treatment of this malignancy.

Our study showed that liposomal curcumin suppresses growth and induces apoptosis in human colorectal cells *in vitro* and in animal models. We also established that liposomal curcumin is internalized into cells to the same extent as free curcumin (Fig. 1), with the added advantage for liposomal curcumin of potentially markedly greater bioavailability because it can be administered i.v. Apoptotic activity was assessed in colon cancer cells by measuring the cleavage of PARP from the native 116 to 85 kDa, which is a hallmark for programmed cell death. There was dose-dependent PARP cleavage after exposure to liposomal curcumin, which was equivalent to that induced by oxaliplatin. We also established synergism between liposomal curcumin and oxaliplatin at a ratio of 4:1 in LoVo cell lines *in vitro* (Fig. 6). In addition, we treated animals bearing human colon cancer xenografts (LoVo and Colo205) with systemically administered liposomal curcumin. Significant tumor growth inhibition without obvious host toxicity was observed (although systematically ruling out toxicity requires more formal animal toxicology studies; Fig. 4); the growth inhibition by liposomal curcumin was greater than that for oxaliplatin in Colo205 xenografts ($P < 0.05$; Fig. 4).

Angiogenesis is integral to tumor growth and metastases. Of interest, in the current study, tumors from animals treated with liposomal curcumin showed an antiangiogenic effect, including attenuation of CD31 (an endothelial marker), VEGF, and interleukin-8 expression (Fig. 5).

There are now numerous publications documenting the potent preclinical antitumor effects of curcumin (4–10). In an early phase I trial in humans, curcumin has shown little, if any, side effects (14). Another pilot study, as well as preliminary results from our phase II study in pancreatic cancer, failed to show any toxicity even in large doses up to 8 g/d given p.o. (14–16). Given the low absorption of p.o. curcumin, we gave liposomal curcumin to animals in i.v. form. We have previously shown that this mode of administration effectively inhibits growth of pancreatic cancer xenografts (8). In both studies, there were no obvious host toxicities, although formal toxicology studies are still needed to rule out side effects in animals.

The molecular basis of tumor inhibition by curcumin is potentially attributable to its effects on transcription factors, apoptotic genes, angiogenesis regulators, and cellular signaling molecules. One of the chief mediators through which curcumin affects many of these pathways is nuclear factor-κB. The role of nuclear factor-κB in pathogenesis of colorectal cancer has been suggested (17). It has been shown to stimulate proliferation of colorectal cancer cells and enhance their survival through the regulation of antiapoptotic genes. A causative role of nuclear factor-κB in colorectal cancer has also been suggested (17) and it regulates the production of cyclooxygenase-2, a factor that is implicated in colon cancer pathogenesis (18).

The antiangiogenic effects of curcumin shown in the current study are consistent with those shown by Arbiser et al. (5), who documented that curcumin suppresses corneal neovascularization in mice. The results are also consistent with another study, which showed that curcumin treatment significantly reduces the tumor neocapillary density and expression of serum VEGF in hepatocellular carcinoma cells (19). Our own previously reported results in pancreatic cancer xenografts also confirm this action of curcumin (8).

Metastatic colorectal cancer is still a terminal illness for most patients. However, survival has increased from a few months to ~2 years (20) mainly because of the introduction of several new agents, each of which has a modest effect. Oxaliplatin, in combination with 5-fluorouracil, has been considered the standard first-line treatment for metastatic colorectal cancer (21). Oxaliplatin is generally well tolerated but can be associated with significant side effects (22) especially after cumulative dosing. This study establishes, for the first time, the comparable or greater growth-inhibitory and apoptotic effects of liposomal curcumin with oxaliplatin, with virtually no side effects for the former, at least in animals. Interestingly, a previous study showed synergism between curcumin and 5-fluorouracil against human colon cancer cells *in vitro*, associated with a 6-fold reduction of cyclooxygenase-2 protein expression (23). In our study, *in vitro* synergism was seen between liposomal curcumin and oxaliplatin at a 4:1 ratio, but there

was no substantial synergy *in vivo*. However, the lack of overlapping side effects suggests that these two drugs could easily be combined.

Colorectal cancer is the second most frequently diagnosed cancer in the United States and incurs significant morbidity and mortality. There are multiple molecular and biochemical events involved in the pathogenesis of this disease. Drugs or combinations of agents affecting multiple pathways are therefore most likely to be effective. Curcumin is a natural plant product with an excellent safety profile that targets multiple signaling pathways. We have shown that it has antitumor and antiangiogenesis effects against colorectal cancer *in vitro* and *in vivo*, and these effects are equivalent or better than those of oxaliplatin, one of the most successful drugs available for treatment of this neoplasm. We are currently developing liposomal curcumin for introduction into the clinical setting.

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