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Curcumin-Loaded Lipid and Polymeric Nanocapsules Stabilized by Nonionic Surfactants: An *In Vitro* and *In Vivo* Antitumor Activity on B16F10 Melanoma and Macrophage Uptake Comparative Study

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Curcumin is a polyphenol obtained from the plant Curcuma longa (called turmeric) that displays several pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial and anti-tumoral activity, but clinical use has been limited by its poor solubility in water and, consequently, minimal systemic bioavailability. We have therefore formulated the drug into nanocarrier systems in an attempt to improve its therapeutic properties. This study evaluates the effect of intraperitoneally administered nanocapsules containing curcumin on subcutaneous melanoma in mice inoculated with B16-F10 cells, and on the cytotoxicity activity against B16-F10 cells *in vitro*. Phagocytic uptake of formulations was also evaluated upon incubation with macrophage J774 cells by fluorescence microscopy. Lipid and polymeric nanocapsules were prepared by the phase inversion and nano-precipitation methods, respectively. The uptake of the lipid nanocapsules prepared using Solutol HS15 was significantly reduced in J774 cells. Curcumin, as free drug or as drug-loaded nanocapsules, was administrated at a dose of 6 mg/kg twice a week for 21 days. Free drug and curcumin-loaded nanocapsules significantly reduced tumor volume ($P < 0.05$ vs. control), but no difference was found in the antitumor activity displayed by lipid and polymeric nanocapsules. This assumption was supported by the *in vitro* study, in which free curcumin as well as loaded into nanocapsules caused significant reduction of cell viability in a concentration- and time-dependent manner.

Keywords: Curcumin, Lipid Nanocapsules, Polymeric Nanocapsules, B16-F10 Melanoma, Anti-tumor Activity, Cytotoxicity, Macrophage Uptake.

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1. INTRODUCTION

Melanoma is the most aggressive form of skin cancer and is characterized by abnormal proliferation of melanocytes that invade the basement membrane.[1] The incidence rate is increasing faster than any other tumor, and the World Health Organization (WHO) estimates 132,000 new cases of cutaneous melanoma per year.[2] Sun exposure, patient phenotype, family history and previous history of melanoma are the major risk factors related to melanoma.[3]

Chemoprevention has been described as an under-explored approach that could significantly decrease morbidity and mortality from this deadly cancer.[4] Chemoprevention strategies mostly imply the use of substances which are able to inhibit or reverse cellular process such as decreased apoptosis, increased proliferation, and cell maturation or differentiation.[5] Particularly, a wide variety of botanicals, mostly dietary flavonoids or phenolic substances including quercetin, resveratrol, and curcumin, have been reported to possess substantial chemopreventive properties because of their antioxidant and anti-inflammatory ability. Concerning skin cancers, laboratory and epidemiological studies have evidenced that

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routine consumption of these polyphenol compounds may favorably provide some antiphotocarcinogenic protection against skin disorders caused by solar UV radiation.[6]

Curcumin is a yellow polyphenol derived from the rhizome of turmeric (*Curcuma longa* L). Extensive research has pointed out its potential in the prevention and treatment of cancer and other diseases. Several studies carried out over the past two decades have indicated that curcumin displays antimutagenic activity and inhibits the radiation- as well as chemical carcinogen-induced neoplastic lesions in many tumor models, including skin cancer.[6] The anticancer potential of curcumin is related to its ability to down-regulate transcription factors NF-κB, AP-1, Erg-1 and MAPK; to down-regulate the expression of COX-2, LOX, NOS, MMP, TNF-α, IL-1β and cyclin D1; to modulate cytochrome P450 function; to down-regulate growth factor receptors and to inhibit the activity of the oncogenes ras, fos, jun and myc.[7–9] *In vivo* studies have demonstrated that curcumin inhibits lung tumor nodule formation in mice inoculated with B16-F10 melanoma cells and increases life span by inhibiting the expression of metalloproteinases.[10, 11] Moreover, the combined treatment with this polyphenol and a prophylactic immune preparation caused substantial growth inhibition of B16-R melanoma cells resistant to doxorubicin and significantly increased the median survival time of animals bearing melanoma tumors.[12]

Despite the potential application of curcumin in the prevention and treatment of skin cancer, this drug exhibits very low bioavailability due to its extremely poor water solubility, which results in low drug absorption in the gastrointestinal tract and fast metabolization in the liver.[13] In addition, curcumin undergoes alkaline hydrolysis at neutral and basic pH, being quickly decomposed in physiological conditions.[14] Among the alternatives to overcome these limitations, the association of curcumin with drug delivery systems has been considered a promising strategy to improve its bioavailability.[13] Recently, some studies have demonstrated the potential of nanostructured drug delivery systems to render hydrophobic agents like curcumin dispersible in aqueous media, thus circumventing the pitfalls of its poor water solubility while maintaining its cytotoxic activity against a number of cancer cell lines.[15–21] However, *in vivo* evaluation of anticancer properties of curcumin-loaded nanocarriers is wanting, despite the great potential of these delivery systems in targeting drugs directly to cancer tissues. On other hand, when administrated intravenously, conventional nanoparticles are rapidly opsonized and removed from circulation by the mononuclear phagocyte system (MPS), mainly represented by the Kupffer cells of the liver and spleen macrophages.[22] Consequently, the contribution of conventional nanoparticles towards increasing anticancer drug efficacy is limited to targeting tumors at the MPS level. Coating nanoparticle surface with hydrophilic polymers such as polyethylene glycol (PEG), so-called Stealth particles, has been shown to reduce clearance by the MPS. As a result, Stealth nanoparticles remained in the circulation for a longer period of time and were also able to extravasate in pathological sites, such as solid tumors located outside the MPS regions.[21, 23]

In previous studies we reported the development of lipid and polymeric nanocapsules containing curcumin with high encapsulation efficiency.[24] Nanocapsules suspensions were prepared using Poloxamer 188 (Pluronic F68) or polyethylene glycol 660-hydroxystearate (Solutol HS15) as hydrophilic surfactants. It is possible that these nanocapsule suspensions exhibit different *in vitro* and *in vivo* antitumor activities related to the presence of PEG chains of Solutol HS15, which are located on the surface of the particles. In fact, the results described in the literature concerning the ability of the nanocapsules made from Solutol HS15 in to escape from the uptake by immune cells are contradictory. *In vivo* biodistribution studies of paclitaxel-loaded lipid nanocapsules shown that the blood distribution of the particles was equivalent to those achieved by other long circulating carriers. Similar results were observed when comparing pharmacokinetics data (MRT, AUC and clearance) of lipid and PLA-PEG nanocapsules, indicating stealth properties of the former, which were explained by the presence of a comparatively higher density of PEG chains on the lipid nanocapsule surface.[25] On the other hand, Béduneau et al. described that lipid systems prepared with PEG 660-hydroxystearate are rapidly removed of blood circulation. In this study, long circulating properties of particles were observed using PEG 1500-hydroxystearate in their production and then stealth properties were considered to be dependent on both chain length and surface density of surfactant.[26]

Considering the above mentioned, this study aims to evaluate and to compare the *in vitro* and *in vivo* activity of curcumin-loaded lipid and polymeric nanocapsules stabilized by nonionic surfactants on a B16-F10 melanoma murine model. Also, *in vitro* studies were carried out on J774 murine macrophage-like cells to verify the ability of the nanocapsules to escape from the uptake by the macrophages.

2. EXPERIMENTAL DETAILS

2.1. Materials

Curcumin and poly (D,L-lactide) (PLA, MW 90,000–120,000) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Soybean hydrogenated lecithin (LIPOID S 75-3N) was provided by Lipoid GmbH (Ludwigshafen, Germany) and castor oil was obtained from Via Farma Importadora Ltda. (São Paulo, Brazil). Polyethylene glycol 660-12-hydroxystearate (Solutol HS15) and Poloxamer (Pluronic F68) were kindly donated by BASF Chemical Company (Ludwigshafen, Germany). Sodium chloride was obtained from Vetec (Rio de Janeiro, Brazil). Except for

the acetonitrile of HPLC grade used in the analysis (Carlo Erba, Milan, Italy), all other reagents and solvents were of analytical grade.

2.2. Cell Lines

A highly metastatic B16-F10 mouse epithelial-like melanoma cell line was donated from Bio-Rio (Rio de Janeiro, Brazil). The cells were cultured in a Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) pH 7.4 supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES in a humidified atmosphere containing 5% CO_2 at 37 °C. For *in vivo* experiments, cells were harvested with a trypsin:EDTA (0.05:0.03 w/v) solution, washed and inoculated into mice in phosphate-buffered saline (PBS; pH 7.4).

For uptake studies, the murine macrophage-like J774 cells were cultured in RMPI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B, in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.3. Preparation of the Nanocapsule Suspensions

2.3.1. Lipid Nanocapsules Suspensions

Lipid nanocapsule suspensions (LNC) were prepared using the phase inversion method.[27] An aqueous phase containing 8.40 g distilled water, 0.75 g NaCl and 4.20 g Solutol HS15 was added to the oil phase containing 1.40 g castor oil, 0.14 g lecithin and 10 mg curcumin, both previously heated to 90 °C, under magnetic stirring. Three temperature cycles alternating from 60 to 95 °C were applied to reach the inversion process. During the last cooling, the suspensions were rapidly diluted with 25.0 ml cold water (approximately 0 °C) and continuously stirred for 30 minutes.

2.3.2. Polymeric Nanocapsules Suspensions

Polymeric nanocapsule suspensions (PNC) were prepared using the interfacial deposition process after solvent displacement.[28] Briefly, 60.0 mg PLA, 0.250 ml castor oil and 2.5 mg curcumin were dissolved in 2 ml of acetone. The resulting solution was added to a 10 ml acetone:ethanol (60:40, v/v) mixture containing 25.0 mg lecithin, and the final volume was adjusted to 12.0 ml. This organic phase was poured into an aqueous phase (26.0 ml) containing 0.375% (w/v) of Pluronic F68 (PNC_P) or Solutol HS15 (PNC_S), maintained under magnetic stirring. The organic solvents were then eliminated by evaporation under reduced pressure, and the final volume of the colloidal suspension was adjusted to 10.0 ml.

Lipid and polymeric nanocapsules suspensions were filtered through 8 μm pore size of filter paper (J-Prolab, São José dos Pinhais, Brazil). The samples were stored protected from light at room temperature before use. Unloaded lipid and polymeric nanocapsule suspensions were prepared and treated in the same manner as the samples.

2.4. Determination of Encapsulation Efficiency and Drug Content

Entrapment efficiency and drug content were estimated after determination of the curcumin concentration in the nanocapsule suspensions by fluorescence spectrophotometry using a previously validated method.[29] The analysis was carried out using a Hitachi F4500 Spectrofluorimeter equipped with a thermostated cell holder set at 25.0 °C. The samples were continuously stirred in a standard 1 cm quartz cell. Both slits of excitation and emission monochromators were adjusted to 5.0 nm. The samples were excited at 397 nm and the emission spectra were recorded from 440 to 600 nm. The relative fluorescence intensities of the samples were measured at $\lambda_{emi} = 508$ nm and compared with that obtained by a curcumin standard solution. A calibration curve for curcumin in acetonitrile was constructed and was linear over the range of 0.1 to 0.7 μg/ml ($R^2 = 0.9957$). The entrapment efficiency (%) was estimated as being the difference between the total concentration of curcumin found in the nanocapsule suspensions after their complete dissolution in acetonitrile and the concentration of drug in the supernatant obtained by suspension ultrafiltration/centrifugation procedure using Microcon Centrifugal Filter Devices with Ultracel YM-100 membrane (nominal molecular weight limit of 100,000 Da, Millipore Corp., USA). The drug content was expressed in μg of curcumin/ml of suspension.

2.5. Determination of Particle Size and Zeta Potential

The mean particle diameter and zeta potential were determined by photon correlation spectroscopy and laser-Doppler anemometry, respectively, using a Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK). The measurements were taken at 25 °C after appropriate dilution of the samples in distilled water. Each size analysis lasted 120 s and was performed with an angle detection of 173°. For measurements of zeta potential (ζ), nanocapsule samples were placed in an electrophoretic cell, where a potential of ±150 mV was established. The ζ potential values were calculated from mean electrophoretic mobility values using Smoluchowski's equation.

2.6. pH Measurements

After preparation, the pH of the nanocapsule suspensions were determined using a pH meter (PHTEK pH100, São Paulo, Brazil), previously calibrated with buffer solutions pH 4.0 and 7.0.

2.7. Evaluation of *In Vitro* Cytotoxicity

In vitro cell viability was assessed using MTT (3-(4,5-dimethiazol-zyl)-2-5-diphenyltetrazolium bromide) assay.[30] Free curcumin or curcumin-loaded lipid and polymeric nanocapsule suspensions were added to B16-F10 cells at different concentrations. Cells were incubated at 37 °C for 24, 48 or 72 hours. After incubation, MTT was added to each well, followed by 3 h incubation. Cells were centrifugated, the supernatant was discarded and the formazan precipitated was dissolved with 100 μL 0.04 N isopropyl alcohol/HCl solution. The absorbance was determined at 540 nm using a microplate reader. Control groups were only plated with cell culture medium and MTT reagent. All assays were performed in triplicate. The statistical analysis was performed using analysis of variance (ANOVA) followed by Bonferoni's post-hoc, using the Graph-Pad Prism (San Diego, CA, USA) software.

2.8. Morphological Assessment of Apoptosis

Apoptosis was verified as described by Geng et al.[31] B16-F10 cells were incubated with free curcumin and curcumin-loaded lipid and polymeric nanocapsule suspensions for 48 hours. Then, acridine orange and ethidium bromide dyes at 10 μg/ml were mixed with cells and the slide was examined under a fluorescence microscope (Olympus BX41) using 40 x objective. Viable cells exhibited green fluorescence (acridine orange staining) whereas apoptotic cells exhibited an orange-red nuclear fluorescence (ethidium bromide staining).

2.9. Macrophage Uptake Studies

Phagocytic uptake and intracellular fate of the fluorescent free curcumin or curcumin-loaded nanocapsules were monitored by fluorescence microscopy upon incubation with J774 cells. Cell uptake was determined in cells grown near to confluence on rounded coverslips in 24-well plates. Upon 60 minutes incubation with free curcumin (previously dissolved in DMSO) or curcumin-loaded lipid or polymeric nanocapsules at 37 °C in the dark, the nanocapsules suspensions were removed, cells were washed three times with phosphate buffered saline (PBS) and coverslips were mounted on a fluorescence microscope. Cell-associated fluorescence of curcumin was monitored with a Nikon Alphaphot 2 YS2 fluorescence microscope (Nikon Corporation Instruments, Japan). Images of the cells were acquired in the emission mode with 40 x objective after exciting the sample at 450 to 490 nm using barrier filter. Fluorescence imaging in the wavelength of 505 nm was collected. In order to quantify the macrophage uptake, image analysis was performed using computer program Scion Image and the mean pixel density of cells was determined by the program Sacci.

2.10. Evaluation of *In Vivo* Antitumor Activity

Male specific pathogen-free C57BL/6 3-month-old mice were used. The animals were kept in a light controlled room (12-hour-light-dark cycle) at a room temperature of 23 ± 2 °C and $60 \pm 10\%$ humidity. Food and water were given ad libitum. All animal studies were carried out in accordance with the procedures outlined in protocol number PP00161/CEUA, for the care and ethical use of animals in research (CEUA/UFSC, Florianópolis, SC, Brazil).

B16-F10 melanoma cells (1×10^6 cells suspended in 100 μl of DMEM medium) were subcutaneously inoculated into the back of male C57BL/6 mice ($n = 8$). Eleven days after inoculation of B16-F10 cells, free curcumin, unloaded or curcumin-loaded nanocapsule suspensions were administered intraperitoneally at a dose of 6 mg/kg, twice a week, corresponding to a total of 4 doses, according to the experimental schedule tested. In order to improve the dispersion of curcumin in the vehicle, the drug was suspended in PBS pH 6.0 containing 0.3% (w/v) of carboxymethylcellulose and Tween 80 0.05% (w/v). The negative control received the cells and PBS while the positive control received the cells and cisplatin, which was given at 1 mg/kg by intraperitoneal route, once a week.

Tumor volumes were measured in the days of treatment throughout 21 days of experiment using a precision calipter and estimated according to the following standard formula: tumor volume (mm^3) = width^2 × length × 0.52, according to Lee et al.[32] The animal body weight was verified in the begging and the end of the experiment. Twenty-one days after inoculation, mice were sacrificed by cervical dislocation and tumors were excised and weighted. The statistical analysis was performed using analysis of variance followed by Dunnet post-hoc.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Lipid and Polymeric Nanocapsule Suspensions

A summary of the physicochemical properties of the nanocapsule suspensions is presented in Table I. Curcumin-loaded polymeric nanocapsule suspensions (Cur-PNC$_P$ and Cur-PNC$_S$) prepared by the nanoprecipitation method displayed a mean particle diameter of around 140 nm, while curcumin-loaded lipid nanocapsule

Table I. Summary of the physicochemical properties of curcumin-loaded lipid and polymeric nanocapsule suspensions.

Sample	Mean diameter (nm) (polydispersity index)	Zeta potential (mV)	Drug content (μg/ml)	pH
Cur-LNC	34.7 (0.247)	−4.5	258.90 ± 0.54	5.95
Cur-PNC$_P$	137.7 (0.208)	−32.9	249.99 ± 1.11	4.65
Cur-PNC$_S$	143.3 (0.191)	−26.4	256.16 ± 0.75	4.60

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(Cur-LNC) produced by the phase inversion process shown a mean size of around 40 nm. Zeta potential values were −32.9 and −26.4 mV for Cur-PNC$_P$ and Cur-PNC$_S$, respectively, and −4.5 mV for Cur-LNC. The high zeta potential values observed for PLA nanocapsules have been attributed to the carboxyl end groups of the polymer, which constitute the polymeric wall of the particles. The negative zeta potential of lipid nanocapsules may be caused by the presence of soybean lecithin phospholipids, which are preferentially located on the nanocapsule surface. However, negative charge was lower than expected, probably due to the masking effect produced by the presence of PEG chains of the Solutol HS15 on the surface of LNC as recently described by Mazzarino et al.[24] Encapsulation efficiency was above 99% for all formulations. These high values can be explained by the poor water solubility of curcumin in the acidic external phase of the nanosuspensions. Drug contents were very close in all preparations, with values around 250 $\mu g \cdot ml^{-1}$.

3.2. Cytotoxic Effect of Free and Encapsulated Curcumin on B16-F10 Melanoma

Data obtained in the *in vitro* cytotoxic study with B16-F10 melanoma cells are shown in Figure 1. Incubation of B16-F10 melanoma cells with free curcumin and curcumin-loaded nanocapsules caused significant reduction of cell viability, in a concentration- and time-dependent manner. After 24 hours of incubation, statistically significant reduction in the number of viable cells was observed for free curcumin at a concentration of $\geq$25 μM, when compared to the control group ($P < 0.05$). At 100 μM concentration, the incubation of cells with free curcumin caused a 75% reduction in the number of viable cells, while a 25% reduction occurred after incubation of Cur-LNC and Cur-PNC$_S$ at same time. On the contrary, after 48 and 72 hours of incubation, Cur-LNC induced a significantly higher cytotoxic effect than free drug at the lowest tested concentration (10 μM), producing a 75% reduction in the number of viable cells ($P < 0.05$). At 10 μM and 72 hours of incubation, the cytotoxic effect in increasing order was Cur-LNC > free curcumin > Cur-PNC$_S$. Cur-PNC$_P$ only exhibited cytotoxic effect at concentration of $\geq$25 μM after 72 hours of incubation, when compared to the control group ($P < 0.05$). The experiments were also performed using the unloaded nanocapsules suspensions (data not shown). The incubation of cells with unloaded nanocapsules caused the reduction of the number of viable cells, suggesting some cytotoxic activity. However, curcumin-loaded nanocapsules reduced the number of viable cells in a significant manner ($P < 0.05$) when compared with the unloaded nanocapsules. The higher cytotoxicity displayed by Cur-LNC may be associated, to some extent, with the small size and lipid structure, which seems favorable in delivering curcumin inside the cells.

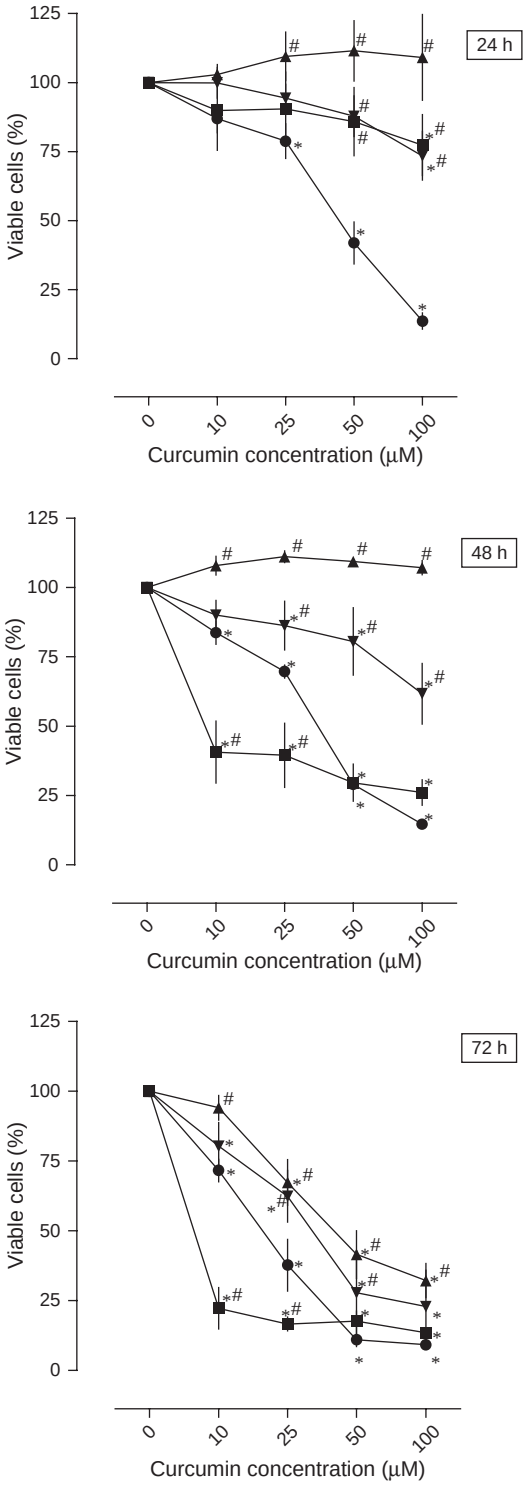

Fig. 1. Cytotoxic effect of free curcumin and curcumin-loaded nanocapsules suspensions on B16-F10 melanoma cells after 24, 48, and 72 hours of incubation. Free curcumin (*circel*), Cur-LNC (*square*), Cur-PNC$_P$ (*triangle*) and Cur-PNC$_S$ (*inversed triangle*). Optical density of control groups was taken as 100% cell viability was confirmed by Trypan Blue exclusion method. $^*P < 0.05$ compared with the control group; $^\#P < 0.05$ compared with free curcumin at the same concentration ($n = 3$).

Previous studies carried out by our research group (data not shown) demonstrated that curcumin is released more quickly from LNC than PNC (77 % and 48% for LNC and PNC, respectively, after 48 hours), due to smaller particle size and thinner shell.[24] Perhaps this nanocapsule property has contributed to the higher cytotoxic effect of LNC when compared to PNC_S and PNC_P. In addition, the nanoencapsulation may have protected the drug from inactivation in the culture medium, since free curcumin is more prone to undergo hydrolytic decomposition at pH 7.4.[33]

The ability of free curcumin and curcumin-loaded nanocapsules to induce apoptosis on B16-F10 melanoma cells was initially screened by using acridine orange/ethidium bromide staining. The treated B16-F10 melanoma cells showed obvious nuclear condensation after 48 hours of treatment. Control cells showed bright green nuclei with uniform intensity and had not taken up ethidium bromide, where the apoptotic cells appeared orange in color (Fig. 2). Based on the cytomorphological changes and cell death described above, the effect of free curcumin or encapsulated curcumin on these cells was indicative of apoptosis.

3.3. Uptake Studies

Uptake of the nanocapsules by macrophages was monitored by morphologic observation of the cells after 60 minutes of incubation (Fig. 3). The yellow fluorescence in the micrographs confirmed the intracellular accumulation of curcumin. Cur-LNC seemed scarcely captured by cells as can be verified by the weak fluorescence in the cytoplasm of the macrophages. On the other hand, polymeric nanocapsules, especially Cur-PNC_P, were more actively phagocyted and accumulated in the cells as was revealed by the bright fluorescence observed. To quantify the macrophage uptake, the fluorescence intensity per cell was related to the number of pixels obtained after image analysis. Fluorescence intensities of Cur-PNC_S, Cur-PNC_p, and free curcumin were around 35, 126, and 5374 times higher than Cur-LNC, respectively. These results suggest that the PEG chains of Solutol HS15 located on the surface of Cur-LNC and Cur-PNC_S reduce the interaction of the particles with J774 cells. In addition, the images showed substantial difference in phagocytic uptake between free and encapsulated curcumin, which indicates that drug uptake can be significantly reduced with the use of colloidal systems.

3.4. *In Vitro* and *In Vivo* Antitumor Activity

To evaluate the effect of free curcumin and curcumin-loaded nanocapsules on tumor growth, C57BL/6 mice were inoculated subcutaneously with B16-F10 melanoma cells (1×10^6 in 100 μl/animal) and treated with the samples. The results of these experiments are summarized in Figure 4. Given twice a week, intraperitoneal injections

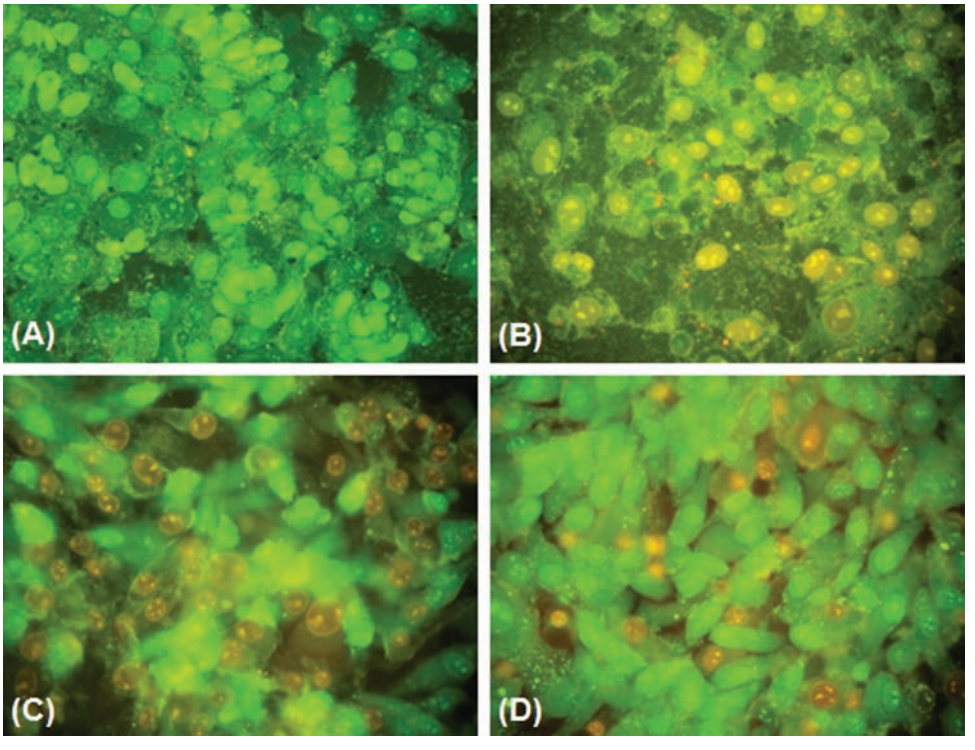

Fig. 2. Cytomorphological changes of B16-F10 melanoma cells induced by free curcumin and curcumin-loaded nanocapsules suspensions upon 48 hours incubation (objective 40 *x*). (A) Control, (B) Free curcumin, (C) Cur-PNC_S and (D) Cur-PNC_P. Viable cells exhibited green fluorescence (acridine orange staining) whereas apoptotic cells exhibited orange-red nuclear fluorescence (ethidium bromide staining).

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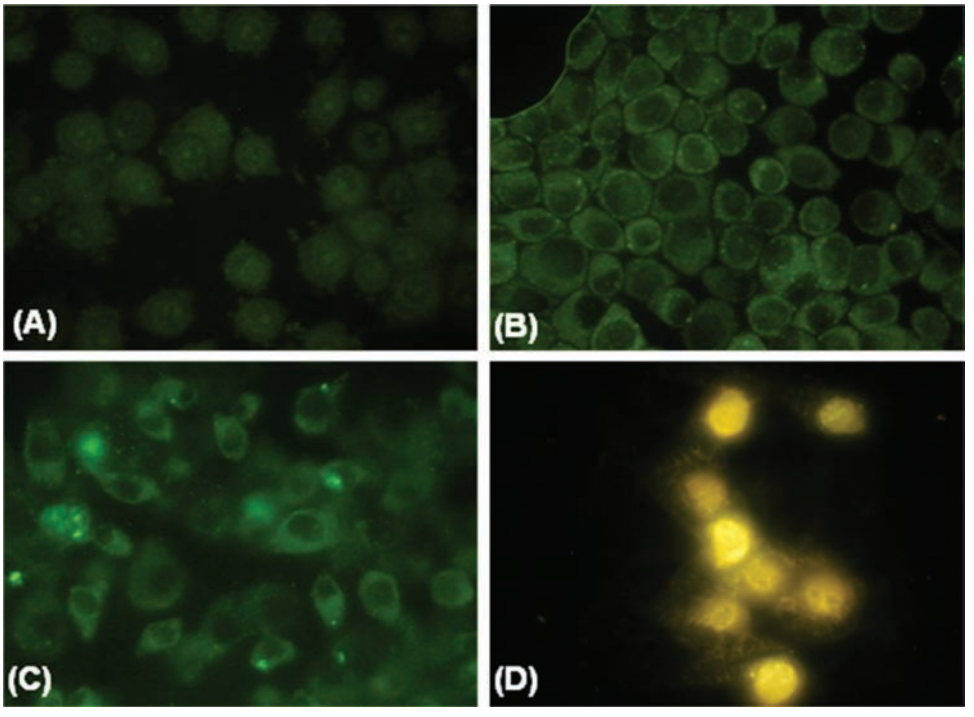

Fig. 3. Fluorescence microscopy images of J774 cells upon 60 minutes incubation in the dark with (A) Cur-LNC, (B) Cur-PNCS, (C) Cur-PNCP and (D) free curcumin (objective 40 *x*). Yellow fluorescence indicates the distribution of curcumin in the macrophages.

of free curcumin or curcumin-loaded nanocapsule suspensions, at a dose of 6 mg/kg, significantly decreased tumor growth rate throughout the 21 days of experiment. By the end of the experiment, tumor volume was significantly inhibited by about 59.6% (1128.4 mm^3), 61.4% (1078.2 mm^3) and 71.3% (801.4 mm^3) after treatment with free curcumin, Cur-LNC and Cur-PNC_S, respectively, when compared to the control group treated with cell culture medium only (2791.0 mm^3) ($P < 0.05$). Cisplatin at a dose of 1 mg/kg, once a week, decreased the tumor volume by 72.4% (770.0 mm^3). No significant difference was found between the tumor volume of the mice that received the free drug and those that received curcumin-loaded nanocapsules, and between the tumor volume of the mice that received unloaded nanocapsules and those that received only saline (negative control group). In a previous study, we had demonstrated that the encapsulation of camptothecin in pegylated PLA-based nanocapsules intraperitoneally administered improves the effectiveness of the drug against the lung metastasis spread in mice inoculated with B16-F10 melanoma when compared with the administration of free drug and non pegylated nanocapsules. This result was attributed to the protection of the drug by nanoencapsulation and to the reduced uptake of particles by macrophages located in the lymph nodes.[34] In the present study, no effect of the nanoencapsulation of curcumin on antitumor activity was verified. In addition, in spite of the differences found in the cytotoxicity and cell uptake exhibited by the colloidal carrier formulations, no improvement in the therapeutic efficacy provided by a possible stealth property of LNC could be confirmed in the *in vivo* studies.

Although both free curcumin and curcumin-loaded nanocapsules demonstrated significant inhibition of melanoma growth, the association of the drug with colloidal systems seems to be beneficial, since it allows the administration of this hydrophobic drug by several routes as an aqueous dispersion, which may result in an increased bioavailability. In earlier studies we demonstrated that the encapsulation of curcumin into both lipid and polymeric nanocapsules increases the concentration of the drug by a factor of up to 46,000 times regarding

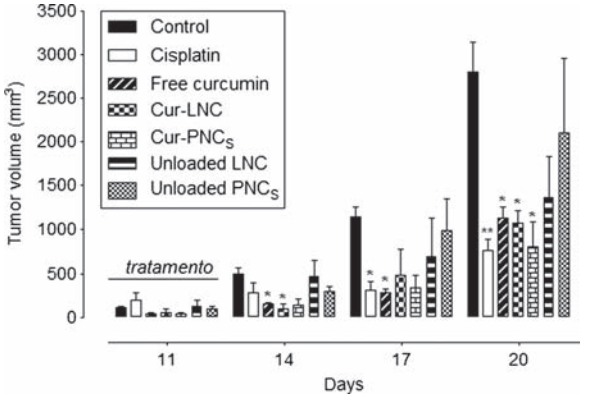

Fig. 4. Effect of free curcumin, unloaded and curcumin-loaded nanocapsules on tumor volume inhibition in mice inoculated with 1×106 B16-F10 melanoma cells. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with control ($n = 8$).

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its aqueous solubility, and besides, it slows down the hydrolytic and photochemical degradation of curcumin.[24] However, pharmacokinetic studies are still needed to comprehend the fate of the curcumin after intraperitoneal administration of the nanocapsules.

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