



Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity *in vitro* and superior bioavailability *in vivo*

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ABSTRACT

Curcumin, a yellow pigment present in the spice turmeric (*Curcuma longa*), has been linked with antioxidant, anti-inflammatory, antiproliferative, anticancer, antidiabetic, antirheumatic, and antiviral effects, but its optimum potential is limited by its lack of solubility in aqueous solvents and poor oral bioavailability. We employed a polymer-based nanoparticle approach to improve bioavailability. Curcumin was encapsulated with 97.5% efficiency in biodegradable nanoparticulate formulation based on poly (lactide-co-glycolide) (PLGA) and a stabilizer polyethylene glycol (PEG)-5000. Dynamic laser light scattering and transmission electron microscopy indicated a particle diameter of 80.9 nm. This curcumin, renamed from hereon “as curcumin (NP)”, was characterized for its biological activity. *In vitro* curcumin (NP) exhibited very rapid and more efficient cellular uptake than curcumin. Estrase staining revealed that curcumin (NP) was at least as potent as or more potent than curcumin in inducing apoptosis of leukemic cells and in suppressing proliferation of various tumor cell lines. When examined by electrophoretic gel shift mobility assay, curcumin (NP) was more active than curcumin in inhibiting TNF-induced NF-κB activation and in suppression of NF-κB-regulated proteins involved in cell proliferation (cyclin D1), invasion (MMP-9), and angiogenesis (VEGF). In mice, curcumin (NP) was more bioavailable and had a longer half-life than curcumin. Overall we demonstrate that curcumin-loaded PLGA nanoparticles formulation has enhanced cellular uptake, and increased bioactivity *in vitro* and superior bioavailability *in vivo* over curcumin.

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1. Introduction

The search for safe, affordable, and efficient agents for prevention and treatment of chronic diseases has led researchers to look in the pharmacopoeia of traditional medicines (TM) for potential therapeutics. Lack of knowledge about the active chemical entity in TM and its mechanism of action has, however, hindered the addition of many TM to modern allopathic medicines. This is not the case with curcumin, a yellow pigment in the spice turmeric (*Curcuma longa*), first isolated in 1815. Its chemical structure, first determined in 1910, is diferuloylmethane [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] [1].

Although the anti-inflammatory activity of turmeric and its role in treatment of various diseases including wound-healing, ulcers, and arthritis is well described in Ayurveda (a science of long life), the work from our laboratory and others has indicated that these activities may be due to the curcumin present in turmeric [2–4]. Extensive research within the last two decades has revealed that curcumin exhibits antioxidant, anti-inflammatory, anti-survival, antiproliferative, anti-invasive and antiangiogenic activity [5]. These effects are mediated in part through the downregulation of various transcription factors including nuclear factor (NF)-κB [6,7], activator protein (AP)-1 [8], hypoxia inducible factor (HIF)-1α [9], and beta-catenin [10]. This leads to the downregulation of various proteins involved in cell proliferation (e.g., cyclin D1), invasion (e.g., matrix metalloproteinase-9; MMP-9) and angiogenesis (e.g., vascular endothelial growth factor; VEGF). Animal studies have revealed that curcumin can prevent carcinogen-induced tumorigenesis and inhibit growth of implanted human tumors [3,11]. Such studies have led to clinical trials of curcumin in patients with

Abbreviations: NF-κB, nuclear factor-kappaB; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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colon cancer [12,13], familial adenomatous polyposis (FAP) [14], pancreatic cancer [15], and multiple myeloma [16].

Unfortunately, these studies have revealed that one of the major problems with curcumin is its oral bioavailability *in vivo*. Traditionally, turmeric is delivered orally as an emulsion in oil or milk; perhaps because of the hydrophobic nature of its bioactive constituents such as curcumin and turmeric oil. Curcumin has indeed been shown to interact with phospholipids [17–20], surfactants [21], proteins [22], and cyclodextrin [23]. Various methods have been tried to enhance curcumin delivery, including its incorporation into liposomes [24,25] and into phospholipid vesicles [26]. The latter was used to deliver curcumin via the intravenous route to bone marrow and splenic macrophages. Another way to solve the problem of lack of water solubility and poor oral bioavailability, is polymer-based nanoparticles [27]. This approach has been used to deliver natural products such as coenzyme Q10 [28], estradiol [29], and ellagic acid [30] and chemotherapeutic agents such as paclitaxel [31], and doxorubicin [32]. In fact a nanoparticle formulation of paclitaxel in which serum albumin is included as a carrier (Abraxane) has been approved for the treatment of breast cancer [33].

Nanoparticle approach has been recently explored for curcumin as well [34]. We have recently shown that silk fibroin-derived curcumin nanoparticles exhibit higher efficacy against breast cancer cells [35]. To further characterize the curcumin nanoparticles, in the present report we describe a biodegradable curcumin nanoparticulate formulation based on poly (lactide-co-glycolide) (PLGA) and a stabilizer polyethylene glycol (PEG)-5000 that exhibit enhanced cellular uptake, and increased bioactivity *in vitro* and superior bioavailability *in vivo* than curcumin.

2. Materials and methods

2.1. Reagents

Curcumin was obtained from Sabinsa Corporation (Piscataway, NJ). Poly (lactide-co-glycolide) (PLGA)-PEG was obtained from Lactal Absorbable Polymers, AL. Ammonium persulphate (APS), ferrous ammonium sulphate (FAS), and N,N'-methylene bis acrylamide (MBA) were obtained from Sigma Chemicals (St. Louis, MO). TEMED was purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was supplied by Atlanta Biologicals (Lawrenceville, GA). Antibodies against cyclin D1 and matrix metalloproteinase (MMP)-9 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Bacteria-derived human recombinant tumor necrosis factor (TNF), purified to homogeneity with a specific activity of 5×10^7 U/mg, was provided by Genentech (San Francisco, CA). Penicillin, streptomycin, Iscove's modified Dulbecco's medium, Dulbecco's modified eagle's medium, RPMI 1640 medium and Live/Dead viability/cytotoxicity kit were obtained from Invitrogen (Grand Island, NY). An anti-vascular endothelial growth factor (VEGF) antibody was purchased from Thermo Scientific (Fremont, CA).

2.2. Cell lines

The cell lines KBM-5 (human chronic myeloid leukemia), Jurkat (human T cell leukemia), DU145 (prostate carcinoma), MDA-MB-231 (breast adenocarcinoma), HCT116 (human colon adenocarcinoma), and SEG-1 (human esophageal adenocarcinoma) were obtained from the American Type Culture Collection (ATCC; Manassas, VA). KBM-5 cells were cultured in Iscove's modified Dulbecco's medium with 15% FBS; Jurkat and DU145 was cultured in RPMI 1640; and HCT116, MDA-MB-231 and SEG-1 cells were cultured in Dulbecco's modified Eagle's medium supplemented

with 10% FBS. Culture media were supplemented with 100 U/mL penicillin and 100 μ g/mL streptomycin.

2.3. Formulation of curcumin loaded nanoparticles

Nanoprecipitation technique was used to prepare the curcumin encapsulated nanoparticles, PLGA-PEG (100 mg) and drug (5 mg) were mixed in acetonitrile (10 mL) added drop wise to an aqueous solution rotating at 5000 rpm containing 0.1% pluronic F-68 as surfactant [36]. The resulting dispersion of nanoparticles was vacuum evaporated to eliminate the organic solvent. The resulting nanoparticles was centrifuged at 15,000 rpm for 15 min and washed with deionized water for three times and freeze-dried with 10% sucrose as a cryoprotectant. The yield of the polymeric nanoparticles was 95% with this protocol.

2.4. Entrapment efficiency (E, %)

The entrapment efficiency (E, %) of curcumin loaded in PLGA-PEG nanoparticles was determined as follows: the nanoparticles were separated from the untrapped free drug using NANOSEP (100 kD cut off) membrane filter and the amount of free drug in the filtrate was measured using spectrophotometer (Spectronic Genesys 5, Thermo Electronic Corp., Waltham, MA). The E (%) was calculated by $E (\%) = ([\text{Drug}]_{\text{tot}} - [\text{Drug}]_{\text{free}}) / [\text{Drug}]_{\text{tot}} \times 100$.

2.5. Dynamic light scattering (DLS) measurements

DLS measurements for determining the average size and size distribution of the polymeric micelles were performed using a Nanosizer 90ZS (Malvern Instruments, Southborough, MA). The intensity of scattered light was detected at 90° to an incident beam. The freeze-dried powder was dispersed in aqueous buffer and measurements were done, after the aqueous micellar solution was filtered with membrane extruder having an average pore size of 0.2 μ m (Lipex, Vancouver, Canada). All the data analysis was performed in automatic mode. Measured size was presented as the average value of 20 runs, with triplicate measurements within each run.

2.6. Transmission electron microscopy (TEM)

TEM pictures of polymeric nanoparticles were taken in a JOEL 1230 transmission electron microscope operating at magnification of 80 kV with $1\text{K} \times 1\text{K}$ digital images captured using an Advanced Microscopy Techniques CCD camera (Danvers, MA). Briefly, a drop of aqueous solution of lyophilized powder (5 mg/mL) was placed on a membrane coated grid surface with a filter paper (Whatman No. 1). A drop of 1% uranyl acetate is immediately added to the surface of the carbon-coated grid. After 1 min excess fluid was removed and the grid surface was air dried at room temperature before loaded in the microscope.

2.7. Scanning electron microscopy (SEM)

The surface morphology of the formulated nanoparticle was measured by scanning electron microscopy (SEM) (EM-LEO 435VP, Carl Zeiss SMT Inc., NY) equipped with 15 kV, SE detector with a collector bias of 300 V. The lyophilized samples were spread over the double-sided conductive tape (12 mm) fixed onto metallic stud.

2.8. Curcumin uptake in cells by fluorescence method

The cellular uptake of curcumin and curcumin nanoparticles in KBM-5 cells, was analyzed by the fluorescence method. In brief,

cells were incubated with Hoechst dye (50 ng/mL; blue fluorescence) for 30 min, and then washed two times with PBS. The washed cells were resuspended in media and then incubated with curcumin and curcumin (NP) for different time intervals. Cells were then examined under a fluorescence microscope (Labophot-2; Nikon, Tokyo, Japan), and images were captured using a Photometrics Coolsnap CF color camera (Nikon, Tokyo, Japan). For vehicle control, cells were incubated with Hoechst dye (50 ng/mL; blue fluorescence) for 30 min, and then washed two times with PBS. The washed cells were resuspended in media and then incubated with 0.01% DMSO (vehicle for curcumin) or PBS for 30 min.

2.9. Apoptosis assay

To compare the apoptotic effects of curcumin and curcumin (NP) in KBM-5 cells, we used a Live/Dead assay kit (Invitrogen, Carlsbad, CA), which determines intracellular esterase activity and plasma membrane integrity. Calcein, a polyanionic, green fluorescent dye, is retained within live cells, and a red fluorescent ethidium homodimer dye enters cells through damaged membranes and binds to nucleic acids but is excluded by the intact plasma membranes of live cells. In brief, cells (5000 per well) were incubated with curcumin and curcumin (NP) for 24 h. Cells were then stained with assay reagents for 30 min at ambient temperature. Cell viability was determined

under a fluorescence microscope by counting live (green) and dead (red) cells.

2.10. Electrophoretic mobility shift assay

To assess NF- κ B activation, we isolated nuclei from cells and carried out electrophoretic mobility shift assays (EMSAs) essentially as previously described. In brief, nuclear extracts prepared from cancer cells (1×10^6 mL⁻¹) were incubated with ³²P-end-labeled 45-mer double-stranded NF- κ B oligonucleotide (4 μ g of protein with 16 fmol of DNA) from the HIV long terminal repeat (5'-TTGTTA-CAAGGGACTTTC CGCTG GGGACTTTC CAGGGA GGCGT GG-3'; bold-face indicates NF- κ B binding sites) for 15 min at 37 °C. The resulting DNA–protein complex was separated from free oligonucleotides on 6.6% native polyacrylamide gels. A double-stranded mutant oligonucleotide (5'-TTGTTACAAC**TA**CT**CA**CTTTC CGCTGCT**CA**CTTTC CAGGGAGG CGTGG-3') was used to evaluate the specificity of binding of NF- κ B to DNA. The dried gels were visualized, and radioactive bands were quantitated using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA) and the ImageQuant software.

2.11. Western blot analysis

Cancer cells were incubated on ice for 30 min in 0.5 mL of ice-cold whole-cell lysate buffer (10% NP-40, 5 mM NaCl, 1 mM HEPES,

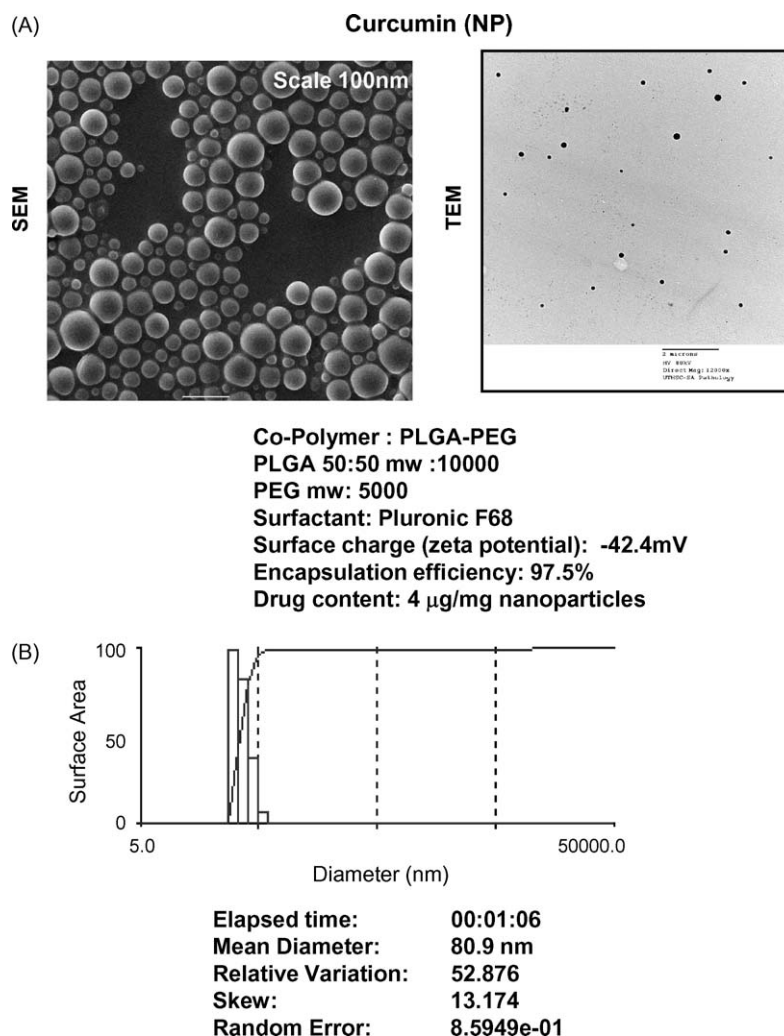


Fig. 1. (A) Curcumin (NP) morphology by scanning electron microscopy (SEM) (left panel) and transmission electron microscopy (TEM) (right panel). (B) Curcumin nanoparticle size: the analysis done by photon correlation spectroscopy.

0.1 mM EGTA, 0.5 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride, 0.2 mM sodium orthovanadate, 1 mM NaF, 2 μ g/mL aprotinin, and 2 μ g/mL leupeptin). Proteins were then fractionated by SDS-polyacrylamide gel electrophoresis, electrotransferred to nitrocellulose membranes, blotted with each antibody, and detected by enhanced chemiluminescence (GE Healthcare, Piscataway, NJ).

2.12. Bioavailability of curcumin and curcumin nanoparticles

The bioavailability of curcumin (NP) was performed in Balb/c mice. The mice were divided into two groups (6 mice in each group), group 1 received curcumin and group 2 received curcumin-loaded nanoparticles. Curcumin or curcumin (NP) were given intravenously (2.5 mg/kg) to each mouse, the blood was collected at different time intervals, serum was separated, and the concentration was determined by HPLC analysis.

3. Results

The focus of this study was to prepare and characterize the curcumin nanoparticles using various methods, with a goal to enhance its bioavailability without loss of biological activity. These nanoparticles were examined for their cellular uptake, for their ability to induce apoptosis and suppress proliferation of tumor cells, for their suppression of NF- κ B and NF- κ B-regulated gene products, and for *in vivo* bioavailability. For most studies, human leukemia KBM-5 cells were used, as the effects of curcumin on these cells have been well described [7]. Throughout the studies, the effects of curcumin (NP) were compared with those of native curcumin.

3.1. Preparation and characteristics of curcumin (NP)

The morphology of curcumin (NP) is shown in Fig. 1A. PLGA-PEG associated curcumin had 97.5% encapsulation efficiency and

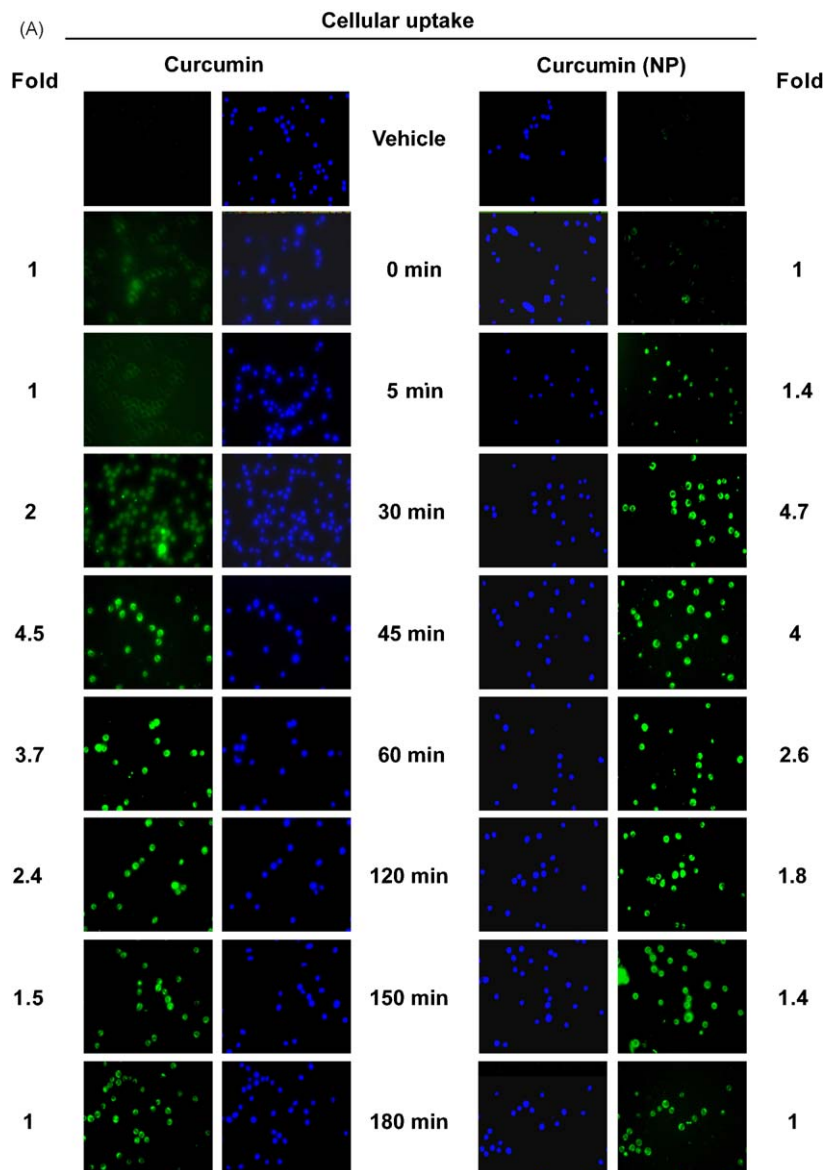


Fig. 2. (A) Cellular uptake of curcumin and curcumin (NP). KBM-5 cells were incubated with Hoechst (50 ng/mL) for 30 min, and then washed two times with PBS, resuspended in media and then incubated with 10 μ M curcumin or curcumin (NP). The cells were harvested at different time intervals and the cellular uptake (green fluorescence) was monitored by fluorescence microscopy as described in Section 2. (B) Disappearance of curcumin and curcumin (NP) from the cells stained with Hoechst (50 ng/mL) for 30 min. KBM-5 (1×10^6) cells were incubated with 10 μ M curcumin or curcumin (NP) for 30 min, washed two times with PBS and again incubated at 37 °C. The cells were harvested at different time intervals and the cellular uptake was determined by fluorescence microscopy as described in Section 2.

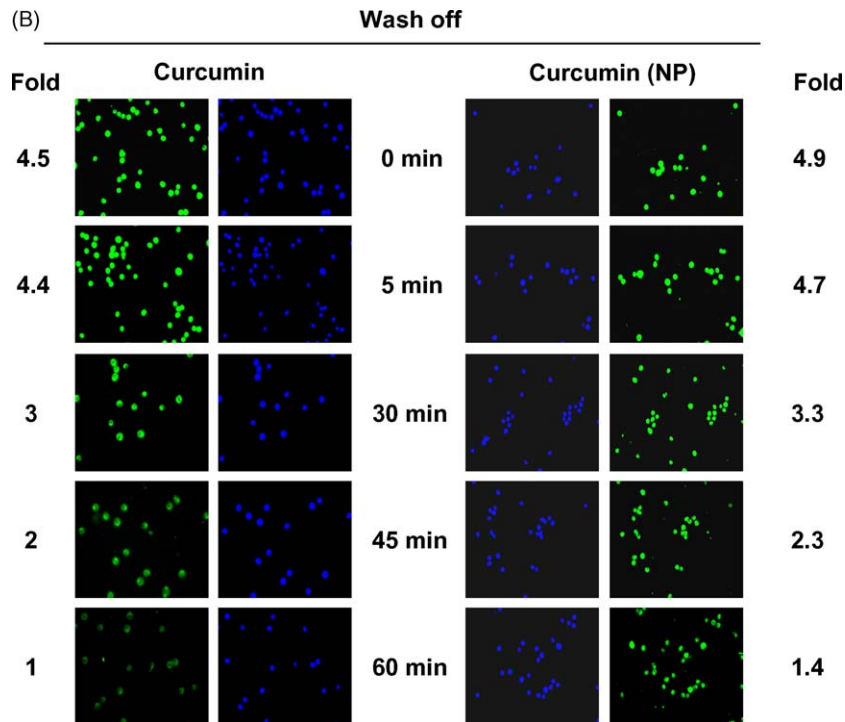


Fig. 2. (Continued).

contained 4 μg curcumin/mg of nanoparticles. Photon correlation spectroscopy showed that the mean diameter of curcumin (NP) was 80.9 nm (Fig. 1B).

3.2. Curcumin (NP) exhibits enhanced cellular uptake

We next investigated the cellular uptake of curcumin and curcumin nanoparticles. For this human chronic myeloid leukemia KBM-5 cells were incubated with curcumin or curcumin (NP) at

10 μM concentration. The cells were harvested at different time intervals and the cellular uptake was determined by fluorescence microscopy. Furthermore, to avoid the background effect from high intensity of fluorescence by curcumin, cells were also stained with Hoechst dye (blue fluorescence). The results showed that curcumin (NP) was taken up as early as 5 min after exposure and reached maximum at 30 min (Fig. 2A). In contrast, earliest uptake of curcumin occurred at 30 min and did not reach maximum even at 180 min (Fig. 2A).

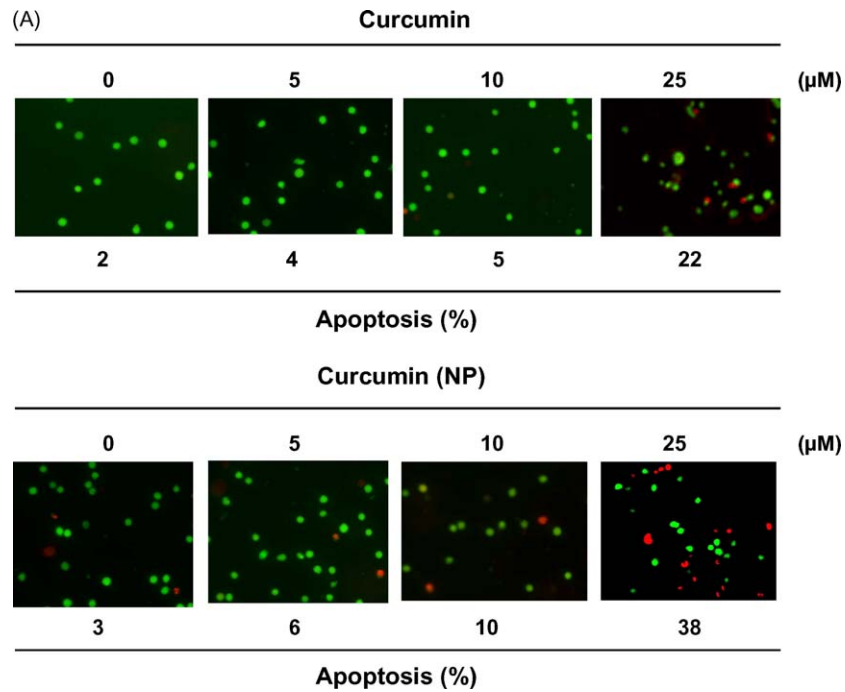


Fig. 3. (A) Curcumin (NP) can induce apoptosis of tumor cells. KBM-5 (1×10^6) cells were incubated with curcumin or curcumin (NP) in indicated concentrations for 24 h. The cells were harvested and stained with Live/Dead assay reagent as per the manufactures protocol as described in Section 2. (B) Curcumin (NP) can inhibit proliferation of tumor cells. Human leukemia (KBM-5 and Jurkat), prostate (DU145), breast (MDA-MB-231), colon (HCT116) and esophageal (SEG-1) cancer cells were incubated with different concentrations of either curcumin or curcumin (NP) in for 72 h and then examined for apoptosis by MTT method. All results were expressed with vehicle control as 100%.

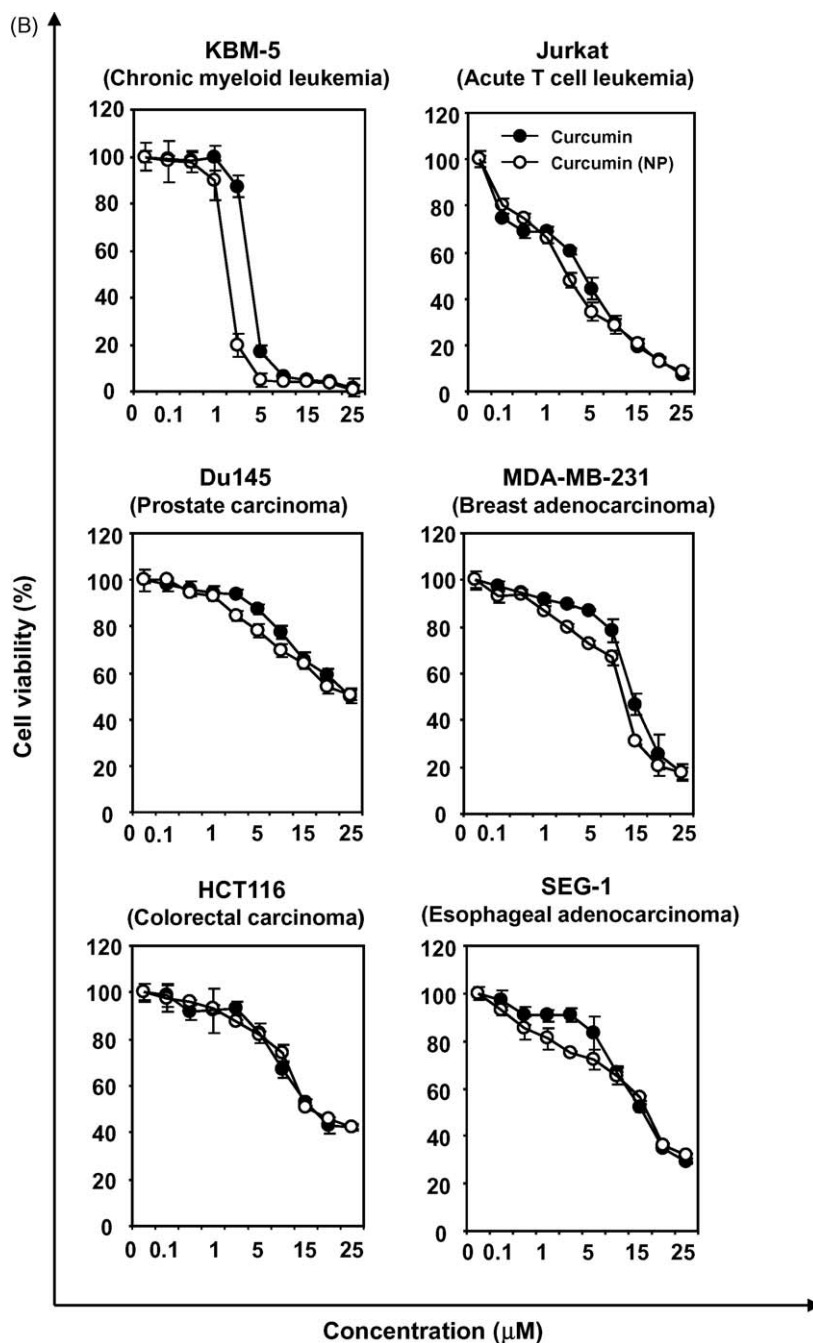


Fig. 3. (Continued).

We also investigated the disappearance of curcumin (NP) from cells after removing the compounds from the medium. For this cells were incubated with curcumin or curcumin (NP) at $10 \mu\text{M}$ concentration for 30 min, washed two times with PBS, and incubated for indicated times at 37°C . The cells were harvested at different time intervals and the cellular uptake was determined by fluorescence microscopy. Interestingly, we found that curcumin (NP) quickly disappeared whereas curcumin disappeared slowly (Fig. 2B).

3.3. Curcumin (NP) induces apoptosis of tumor cells

Next, we investigated the ability of curcumin (NP) to induce apoptosis in human cancer cells. When human KBM-5 cells were

incubated with different concentrations of curcumin or curcumin (NP) for 24 h, esterase staining showed a dose-related apoptotic response (Fig. 3A). At $25 \mu\text{M}$ curcumin (NP) was somewhat more active than curcumin.

3.4. Curcumin (NP) inhibits proliferation of tumor cells

Human leukemia (KBM-5 and Jurkat), prostate (DU145), breast (MDA-MB-231), colon (HCT116) and esophageal (SEG-1) cancer cells were incubated with different concentrations of either curcumin or curcumin (NP) for 72 h and then examined for apoptosis by MTT method. The results in Fig. 3B show that curcumin (NP) suppressed cell proliferation dose-dependently at least as potently as native curcumin.

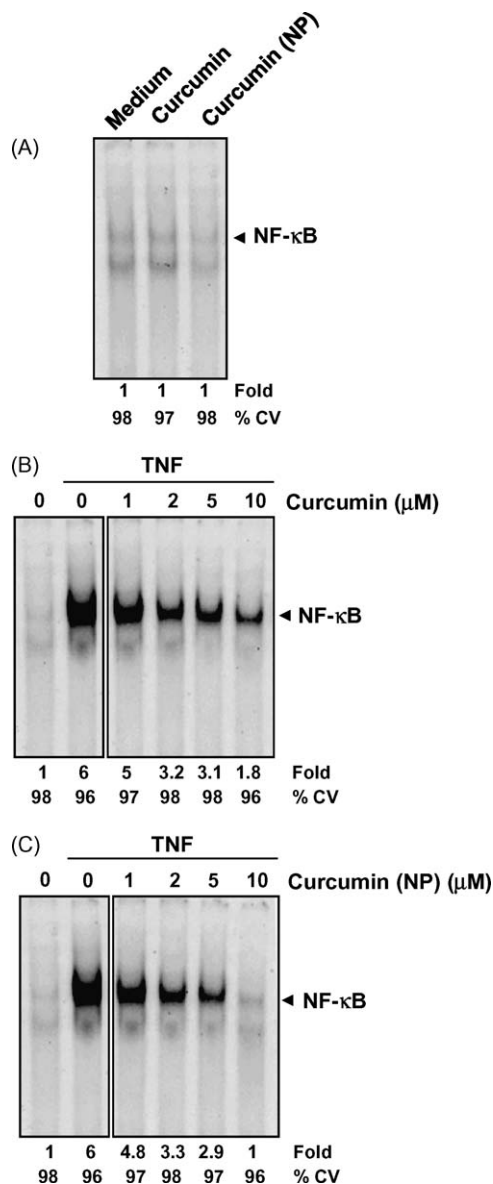


Fig. 4. (A) Curcumin or curcumin (NP) do not induce NF-κB activation in KBM-5 cells. KBM-5 (2×10^6) cells were treated with indicated concentrations of curcumin or curcumin (NP) for 4 h. Nuclear extracts were prepared and the NF-κB activity was examined by EMSA. (B and C) Curcumin (NP) is more potent than regular curcumin in inhibiting TNF-induced activation of NF-κB. KBM-5 (2×10^6) cells were treated with indicated concentrations of curcumin or curcumin (NP) for 4 h. The cells were then incubated with TNF 0.1 nM for 30 min and analyzed for NF-κB activity by EMSA. The cytotoxicity was examined using trypan blue exclusion method.

3.5. Curcumin (NP) suppresses NF-κB activation

Suppression of NF-κB is one of the major activities assigned to curcumin. Whether curcumin (NP) by itself can induce NF-κB, was examined. KBM-5 cells were treated with the indicated concentrations of curcumin (NP) for 4 h, and nuclear extracts were prepared and analyzed for NF-κB activity by EMSA. Neither curcumin nor curcumin (NP) alone activated NF-κB (Fig. 4A). TNF-induced activation of NF-κB, however, was inhibited by curcumin in a dose-dependent manner (Fig. 4B), which was also the case for curcumin (NP) (Fig. 4C). Curcumin (NP) was more potent than curcumin in this effect. Under the conditions, curcumin or curcumin (NP) had no significant effect on cell viability, indicating

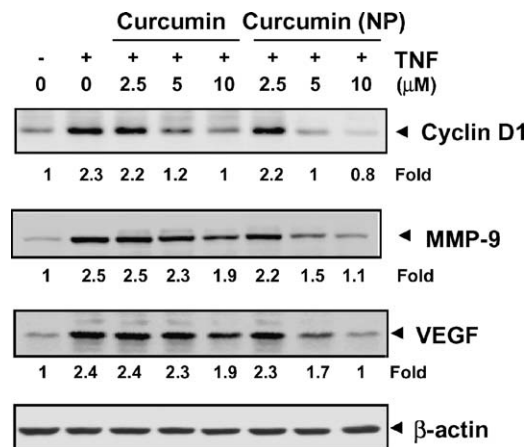


Fig. 5. Curcumin (NP) is more potent than curcumin in inhibiting TNF-induced expression of NF-κB-regulated genes. KBM-5 (1×10^6) cells were co-incubated with TNF (1 nM) and curcumin or curcumin (NP) (10 μM) for indicated time intervals. The cells were harvested and the expression of cyclin D1, MMP-9, and VEGF analyzed by western blot. β-Actin was used as a loading control.

that suppression of NF-κB activation was not due to loss of cell viability.

3.6. Curcumin (NP) inhibits the expression of NF-κB-regulated gene products

To compare the potency of curcumin (NP) and curcumin in inhibiting TNF-induced expression of NF-κB-regulated gene products, we co-incubated KBM-5 cells with TNF and curcumin or with TNF and curcumin (NP) for different time intervals and then examined for cyclin D1 (cell proliferative), MMP-9 (invasion) and VEGF (angiogenesis) gene products. As shown in Fig. 5, curcumin (NP) suppressed the expression of all these gene products more potently than curcumin.

3.7. Curcumin (NP) is more bioavailable than curcumin

One of the major reasons for making curcumin (NP) to examine its effect on bioavailability *in vivo*. For this, mice were intravenously administered either curcumin or curcumin (NP) (2.5 mg/kg), blood was collected at different time intervals, and curcumin concentration was determined by HPLC analysis. Results in Fig. 6

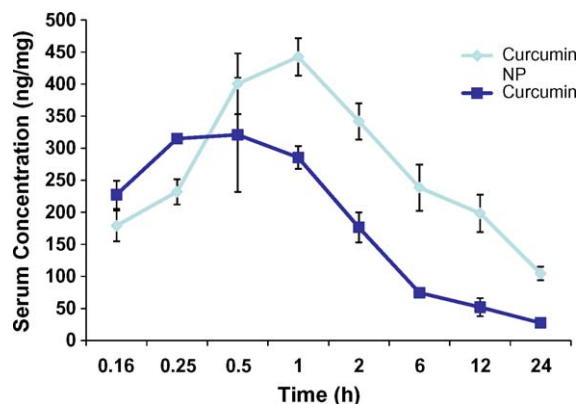


Fig. 6. Bioavailability of curcumin and curcumin (NP). The mice were divided into two groups (6 mice in each group), group 1 was given curcumin and group 2 was given curcumin (NP). Curcumin and curcumin (NP) were administered intravenously (2.5 mg/kg) and the blood was collected at different time intervals. Serum was separated and the concentration of curcumin and curcumin (NP) were determined by HPLC analysis.

clearly show serum levels of curcumin were almost twice as high in the case of curcumin (NP) administration as it was curcumin administration. In addition, half-life of curcumin (NP) was substantially longer than that of curcumin.

4. Discussion

Curcumin, a yellow pigment from turmeric, has been associated with antioxidant, anti-inflammatory, antiproliferative, anticancer, antiangiogenic, and antidiabetic activities. However poor water solubility and limited oral bioavailability are the major roadblocks in its development as a therapeutic for cancer and other chronic diseases. In the current report we prepared a biodegradable nanoparticle formulation of curcumin based on nanoprecipitation technique using PLGA-PEG and tested its bioavailability and effects on cells growth. This method has been used to encapsulate a wide variety of hydrophobic drugs including coenzyme Q [28], taxol [31,37], estradiol [29], ellagic acid [30], and camptothecin [32]. Our results are in agreement with Bisht et al. [34] who made nanoparticles of curcumin. However, these authors reported very limited information about its biological attributes *in vitro* and no information on its bioavailability *in vivo*.

We found that curcumin (NP) had much higher cellular uptake *in vitro* than that of curcumin. Its rate of disappearance from the cells, however, was very similar. Increased cellular uptake of curcumin (NP) is in agreement with Bisht et al.'s report [34]. We also found that curcumin (NP) was more potent in inducing apoptosis in tumor cells. One possible cause for higher activity could be higher cellular uptake of curcumin (NP).

Curcumin (NP) inhibited the growth of a wide variety of tumor cells in a dose-dependent manner. This effect was comparable with that of curcumin. Why curcumin (NP) did not show significantly higher efficacy than curcumin is not clear. Because apoptosis was examined during a short time (24 h) and antiproliferative effects during a long time (72 h), it is possible that in short-term assays curcumin (NP) is more active than curcumin but not in long term assays, perhaps due to the differential cellular uptake.

Curcumin (NP) was more active than curcumin in suppressing NF- κ B activation. This difference again could have been due to differential uptake. Bisht et al. [34] did not examine TNF-inducible NF- κ B but showed that curcumin (NP) could suppress constitutive NF- κ B in pancreatic cancer cells.

We found that curcumin (NP) was also more active than curcumin in suppressing the expression of TNF-regulated expression of cyclin D1, MMP-9 and VEGF; most likely again due to enhanced cellular uptake. These results imply that curcumin (NP) may be superior to curcumin as an antitumor, anti-invasive and antiangiogenic agent. The results are in agreement with a report that curcumin (NP) downregulates IL-6, another NF- κ B-regulated gene product [34].

Finally, our results also show that in animals curcumin (NP) is more bioavailable and has a substantially longer half-life. In agreement with Bisht et al. [34], we found that curcumin (NP) was not toxic to the animals. Formation of polymeric micelles of curcumin with copolymer micelles of methoxy poly(ethylene oxide)-b-poly(epsilon-caprolactone) (MePEO-b-PCL), has been shown to increase its half-life by 162-fold [38]. These results are in agreement with a report that showed that curcumin formulated with phosphatidyl choline (PC), when given to animals, had a peak plasma levels and area under the plasma concentration time curve (AUC) values were higher than the unformulated curcumin [20]. They also showed that liver levels of curcumin were higher after administration of PC-curcumin as compared to unformulated curcumin. In contrast, curcumin concentrations in the gastrointestinal mucosa after ingestion of PC-curcumin were somewhat lower than those observed after administration of unformulated

curcumin. Curcumin has been conjugated with numerous carriers including phospholipids [18], cyclodextrin [23], phosphatidyl choline [20], and liposomes [25,39], but very little information is available about its biological activities. It has been shown that binding states of curcumin to all these carriers is typical of amphipathic drugs [40]. More studies are now needed to investigate the *in vivo* efficacy of curcumin (NP) against various types of cancer. Although curcumin is pharmacologically quite safe in human [1,11,41], the efficacy of curcumin (NP) remains to be determined. Polymers such as albumin-bound paclitaxel (Abraxane) have been approved for human use for the treatment of cancer [33]. Overall, our results suggest that curcumin (NP) is likely to have great potential as a therapeutic, but more studies are required.

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