

## Research Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery

Guest Editor: Sanyog Jain

# In Vitro Cytotoxicity and Bioavailability of Ginsenoside-Modified Nanostructured Lipid Carrier Containing Curcumin

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**Abstract.** Our aim was to investigate the cellular uptake, *in vitro* cytotoxicity and bioavailability of ginsenoside-modified nanostructured lipid carrier loaded with curcumin (G-NLC). The formulation was prepared by melt emulsification technique, in which water was added to the melted lipids and homogenized to give a uniform suspension of NLC (without ginsenoside) and G-NLC. Cellular uptake of curcumin in two colon cancer cell lines (HCT116 and HT29) was increased when administered using both NLC and G-NLC compared to control (curcumin dissolved into DMSO) as measured by fluorescence microscopy. Ginsenoside modification resulted in 2.0-fold and 1.4-fold increases in fluorescence intensity in HCT116 and HT29 cell lines, respectively, compared to plain NLC. *In vitro* cytotoxicity (assessed by MTT assay) had a dose-dependent relationship with curcumin concentration for both NLC and G-NLC. Although G-NLC was taken up more readily in HCT116 cells, ginsenoside modification did not produce a significant increase in cytotoxic effect; a significant increase was observed in HT29 cells. Oral administration of G-NLC in ten colon cancer patients produced an appreciable plasma level of unbound curcumin (2.9 ng/mL). In conclusion, introduction of ginsenoside into NLC enhanced the cellular uptake and cytotoxicity of curcumin as well as its oral bioavailability, and this strategy can be used to improve clinical outcomes in the treatment of colon cancer with similar genotype to HT29.

**KEY WORDS:** curcumin; cellular uptake; *in vitro* cytotoxicity; ginsenoside modification; colon cancer.

## INTRODUCTION

Curcumin, the major active constituent of turmeric plant (*Curcuma longa*), is a natural polyphenol compound that has been used for centuries as a traditional medicine in India, China, and other Asian countries. Extensive research has shown that it is beneficial as a treatment for a variety of diseases (1–5). However, the major problems that limit its applicability are poor solubility and absorption, which, in

turn, reduce its bioavailability. As curcumin is practically insoluble in water, improving its bioavailability is a difficult challenge. Although several *in vivo* studies have been conducted and some curcumin products have seen commercial use, curcumin still suffers from poor pharmacokinetic properties limiting its therapeutic application in human subjects. This may be due to several factors such as age, health, sex, and race (6–9). In addition, curcumin undergoes rapid metabolism, with only its metabolite curcumin glucuronide and the curcumin sulfates detected in plasma after administration (10,11).

Possible solutions include the use of an adjuvant or excipient to overcome these problems. Multifold increase in curcumin plasma concentration was reported when administered along with piperine (12). Here, we used ginsenoside in the composition of a nanostructured lipid carrier loaded with curcumin (NLC) to improve the bioavailability of curcumin. It is widely known that cholesterol and ergosterol in the cell membrane of eukaryotic cells contribute to stabilizing the phospholipids. As such, we hypothesized that ginsenoside, a steroid glycoside, could exert beneficial effect on the stabilization of the NLC (13,14). We also hypothesized that

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ginsenoside may play a role in the absorption process through the enterocyte cell membrane and, consequently, enhance the bioavailability of the incorporated curcumin.

Previously, we developed a monoolein (MO)-based liquid crystalline nanoparticle dispersion loaded with curcumin, and found that the dispersion was very stable in terms of size and surface charge evolution of the nanoparticles (15). We subsequently found that, even in the absence of curcumin, the dispersion began to show cytotoxicity at a nanoparticle concentration of approximately 20  $\mu\text{g/mL}$  (16). Therefore, we report here our attempt to circumvent this cytotoxicity by preparing a liquid crystalline nanoparticle consisting of phospholipids, replacing MO (17).

In this study, we developed a ginsenoside-modified nanostructured lipid carrier (G-NLC) dispersion and found that it enhanced the stability of the dispersion and improved the bioavailability of curcumin in rats. Specifically, we aimed to investigate the *in vitro* cytotoxicity of the G-NLC dispersion and its bioavailability in patients with colon cancer. We initially evaluated the *in vitro* cytotoxicity of G-NLC, with or without curcumin loading, towards two colon cancer cell lines (HCT116 and HT29). We also investigated cellular uptake of curcumin in these colon cancer cell lines using fluorescence microscopy. Furthermore, we administered the G-NLC dispersion to patients with unresectable metastatic colon cancer to determine its bioavailability in humans.

## MATERIALS

Curcumin was purchased from Sigma-Aldrich (St Louis, USA). Lecithin and lysophosphatidylcholine were obtained from Lipoid (Ludwigshafen, Germany). Refined hydrogenated soybean oil (HBO) was purchased from Lotte Foods (Seoul, Korea). Ginsenoside was prepared by fermentation of ginseng saponin obtained from The Zone PHC (Chubunmyeon, Korea) for 7 days using *Saccharomyces cerevisiae* and a subsequent purification procedure. The ginsenosides present in the fermented ginseng saponin were composed of ginsenoside Rg1, 39.99%; Rd, 14.05%; F2, 13.79%; protopanaxadiol, 11.55%; Rg3, 7.95%; compound K, 5.75%; protopanaxatriol, 1.83%; and other minor components. Human colon cancer cell lines (HCT116 and HT29) were purchased from the American Type Culture Collection (Manassas, USA). RPMI-1640 culture media were purchased from Gibco® (Thermo Fisher Scientific, Waltham, USA). All other chemicals were of reagent grade and were used without further purification.

## METHODS

### Preparation of Curcumin-Loaded Nanostructured Lipid Carrier

NLC and G-NLC were prepared by melt emulsification technique, as described in an earlier paper (17), with slight modification. Briefly, all the ingredients specified in Table I, except water, were added to a round-bottom flask and solubilized using a magnetic stirrer at 60°C. Once the mixture was solubilized, the ethanol in the mixture was evaporated using a rotary evaporator at 60°C. Pre-heated distilled water (60°C) was then added and the mixture was immediately

homogenized using a homogenizer at 24,000 rpm for 5 min. The dispersion thus obtained was cooled down to room temperature and stored in a refrigerator (4°C) until usage.

### Characterization of Curcumin-Loaded Nanostructured Lipid Carrier

The particle size distribution and polydispersity index (PDI) of the NLC and G-NLC dispersion were determined by dynamic light scattering using a Zetasizer Nano S90 (Malvern Instruments, UK). The dispersion sample was appropriately diluted with distilled water (18.2  $\text{M}\Omega\text{ cm}^{-1}$ ) and measured at 25°C using a disposable cuvette. The measurement was performed in triplicate with three sets of 15 runs, and the mean values of size and PDI were measured. Morphology was examined by scanning electron microscopy (SEM) using a JSM-7500F (JEOL, MA, USA) and the image was taken at an accelerating voltage of 15 kV.

### Cell Culture

HCT116 and HT29 cells were grown in RPMI media containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in a humidified atmosphere with 5%  $\text{CO}_2$ .

### Cellular Uptake of Curcumin

The cellular uptake of curcumin dissolved in DMSO (C-DMSO) and curcumin-loaded nanostructured lipid carriers (NLC, G-NLC) by the human colon cancer cell lines (HCT116 and HT29) was studied using fluorescence microscopy. For fluorescence microscopy, HCT116 and HT29 cells were seeded in a 30-mm plate at a density of  $2 \times 10^4$  cells and incubated for 24 h at 37°C to allow attachment. Cells were then incubated with RPMI-1640 medium plus C-DMSO, NLC, or G-NLC to allow the curcumin and/or nanoparticles to be taken up by the cells, with a curcumin concentration of 5  $\mu\text{g/mL}$  at 37°C (DMSO concentration in the culture medium, 0.5%). After treatment for 30 min, the cells were rinsed three times with PBS to wash out the nanoparticles remaining on the surface of the cells. Fresh PBS was added to the plate, and the cells were visualized by fluorescence microscopy (Axio Imager.Z1; Carl Zeiss Meditec AG, Jena, Germany).

In measuring the fluorescence intensity, HCT116 and HT-29 ( $2 \times 10^5$ ) cells were seeded on 35-mm dishes and grown to 90% confluence under cell culture conditions. Cells were then incubated with C-DMSO, NLC, and G-NLC (5  $\mu\text{g/mL}$  as curcumin) for different time durations (30 min or 60 min). Cells were washed twice with PBS to remove unbound nanocarriers. The cells were then trypsinized and centrifuged at 1500 rpm for 3 min, after which the pellet was resuspended in 500  $\mu\text{L}$  of phosphate-buffered saline (PBS). The suspended cells were analyzed by flow cytometry FACS Calibur™ (BD Bioscience, USA) at excitation (495 nm) and emission (519 nm) wavelengths to detect curcumin. No fluorescence was observed when the cells were incubated with vehicle without curcumin.

**Table I.** Composition of Curcumin-Loaded Nanostructured Lipid Carrier

Composition code	Ginsenoside (g)	Curcumin (g)	Lecithin (g)	LPC (g)	HBO (g)	Ethanol (g)	Distilled water (g)	Total (g)
NLC	0	0.1	2.0	0.2	0.2	1.0	27.4	30.0
G-NLC	0.5	0.1	2.0	0.2	0.2	1.0	26.9	30.0
NLC without curcumin	0	0	2.0	0.2	0.2	1.0	27.5	30.0
G-NLC without curcumin	0.5	0	2.0	0.2	0.2	1.0	27.0	30.0

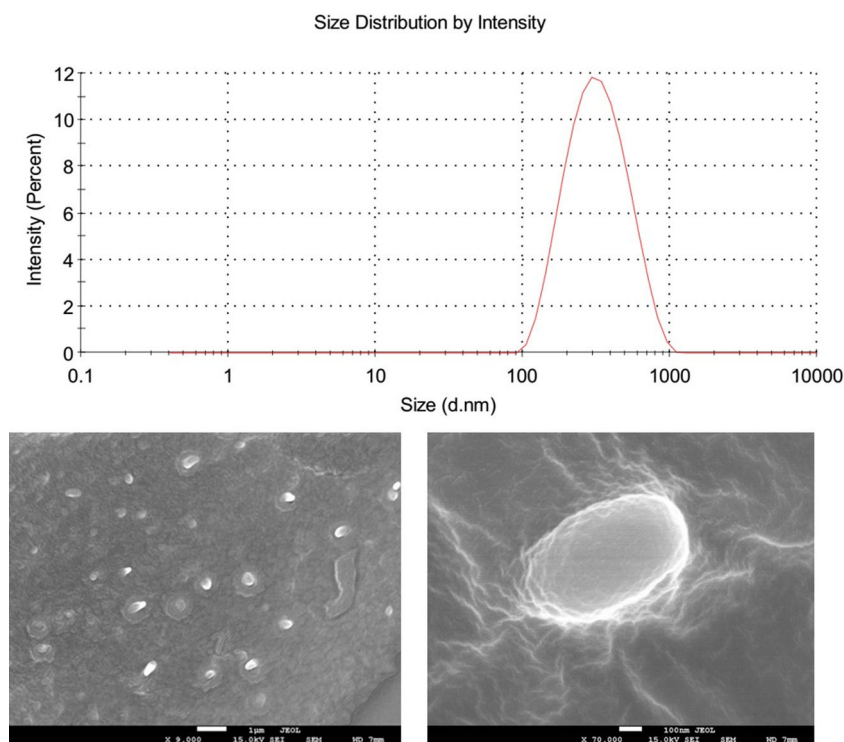
*HBO*, hydrogenated soybean oil; *G-NLC*, ginsenoside-modified nanostructured lipid carrier loaded with curcumin; *LPC*, lysophosphatidylcholine; *NLC*, nanostructured lipid carrier loaded with curcumin

### In Vitro Cytotoxicity

*In vitro* cytotoxicity of G-NLC on cancer cell lines HCT116 and HT29 was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as described elsewhere in an earlier paper (18). The cells were seeded in a 96-well plate at a density of  $1 \times 10^5$  cells and incubated for 24 h at 37°C to allow cell adhesion. The cells were then treated with different concentrations of C-DMSO (curcumin in DMSO), NLC, and G-NLC for 48 h. The medium was removed and 100  $\mu$ L of MTT solution (1 mg/mL) was added to each well, following which, the cells were incubated for 2 h at 37°C. The cells were then washed with PBS and 100  $\mu$ L of DMSO was added to dissolve the formazan crystals formed by the living cells. The plate was shaken for 5 min and the absorbance was measured at 570 nm using a microplate reader. All assays were repeated six times.

### Clinical Study

Ten patients (6 females and 4 males) with unresectable metastatic colon cancer were recruited; all patients were on a standard chemotherapy regimen of Avastin/FOLFIRI (folinic acid, 5-fluorouracil, irinotecan with bevacizumab). Additional inclusion criterion was patients older than 20 years with histologically confirmed adenocarcinoma at the colon. Exclusion criteria were patients who received adjuvant chemotherapy within 6 months, patients with peritoneal metastasis, or patients planning radical surgery for metastatic lesions. The study protocol was approved by the Institutional Review Board of Gachon University Gil Medical Center (GAIRB2015-87), and written informed consent was obtained from patients before their entry into the study. Patients were educated on the food sources of curcumin and asked to avoid any foods containing curcumin before administration of G-NLC. A high-fat meal was



**Fig. 1.** Particle size distribution and SEM images of ginsenoside-modified nanostructured lipid carrier

designed and recommended to patients in order to slow down gastric emptying time. Curcumin was administered orally as a G-NLC dispersion (AJP0513<sup>TM</sup>, 30 mL drink formula in a brown bottle containing 100 mg curcumin) manufactured by GMP-certified Ajou Pharm. Co., Korea, two times daily immediately after a meal in the morning and evening for 12 weeks. Composition of each dispersion is listed in Table I. At the sixth and the twelfth weeks, blood samples were collected after 30 min of administration in a heparinized tube and stored at  $-80^{\circ}\text{C}$  until LC-MS analysis.

### LC-MS/MS Analyses

Levels of curcumin in patient blood were determined by an LC-MS/MS system comprising an Agilent LC 1100 series (Agilent Technologies, CA, USA) binary pump, a vacuum degasser, and an auto-sampler system connected to a 6490 triple quadrupole MS equipped with electrospray ionization (ESI) source, with Agilent jet stream technology. Chromatographic separation was performed on a Zorbax Extend-C18 ( $1.0 \times 150$  mm,  $3.5 \mu\text{m}$ ) column. The column temperature was maintained at  $28^{\circ}\text{C}$ , and the auto-sampler temperature was set at  $4^{\circ}\text{C}$ . Sample solutions ( $4 \mu\text{L}$ ) were injected and the analytes were eluted using 0.1% formic acid in acetonitrile and 0.1% formic acid in water (50:50, v/v) at a flow rate of 0.1 mL/min. The isocratic separation run time was 10 min. Hesperetin was used as an internal standard. The operational parameters were as follows: argon as a collision gas, gas flow of 14 L/min, source temperature of  $250^{\circ}\text{C}$ , and collision energies of 14 and 24 eV for curcumin and hesperetin, respectively.

Quantification was performed using a multiple reaction monitoring method by observing the transitions of precursor-to-product ions  $m/z$  369.1  $\rightarrow$  285.1 for curcumin,  $m/z$  543.2  $\rightarrow$  216.9 for curcumin glucuronide, and  $m/z$  303.1  $\rightarrow$  153 for hesperetin. Mass data were collected and processed using the MassHunter QQQ Qualitative and Quantitative software (Version B.04.00, Agilent Technologies, USA).

Calibration standards, QC samples, and human plasma samples were extracted with acetonitrile using a protein precipitation method. Specifically, before the analysis, the plasma sample was thawed to room temperature. A volume of  $10 \mu\text{L}$  acetonitrile with standard working solution was added to  $100 \mu\text{L}$  final volume of the plasma sample in a 1.5-mL Eppendorf tube. Next,  $200 \mu\text{L}$  acetonitrile with an internal standard (5 ng/mL) was added for protein precipitation after vortexing for 1 min, followed by centrifugation at 14,000 rpm for 15 min at  $4^{\circ}\text{C}$ . The supernatant was separated and transferred to an auto-sampler vial insert and  $4 \mu\text{L}$  of the sample was injected into the LC-MS/MS system. Lower limit of quantification (LLOQ) was found to be 1 ng/mL for curcumin. Blank plasma (matrix) did not show any peak at the retention times of the target and internal standard.

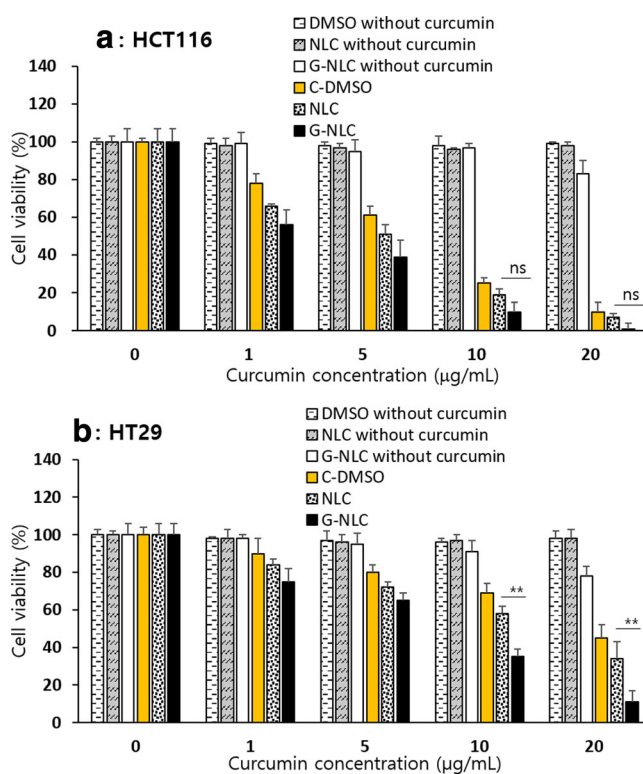
### Statistical Analysis

All data were analyzed using Student's *t* test in Microsoft Office Excel 2010. Data are presented as mean  $\pm$  standard deviation. A value of  $p < 0.05$  was considered statistically significant.

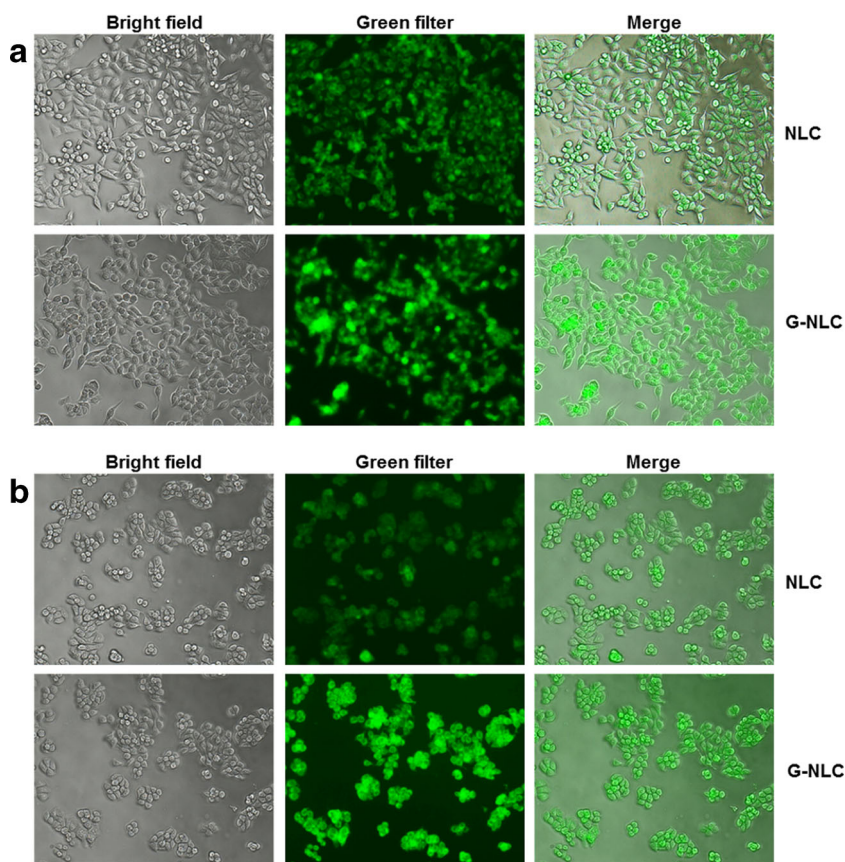
## RESULTS

NLC and G-NLC were prepared by the melt emulsification technique. Each mixture was heated and mixed constantly until all the ingredients were solubilized. Water was added and high shear homogenization was applied at 24,000 rpm for 5 min to achieve uniform dispersion. The dispersion appeared as a thick mango-yellow colored liquid, with a mean particle size of  $340.6 \pm 33.64$  nm (Fig. 1) and a polydispersity index (PDI) of  $0.172 \pm 0.063$ . SEM imaging revealed that thin thread-shaped materials were adhered on the surface of the nanoparticle, which was also found in the previous study with transmission electron microscopy (17).

Figure 2 depicts the *in vitro* cytotoxicity of different concentrations of curcumin against human colon cancer lines HCT116 and HT29. Curcumin exhibited growth inhibition in a dose-dependent manner in both cell lines. Inhibition by G-NLC was significantly superior to that by NLC at  $10 \mu\text{g/mL}$  or higher in HT29 line ( $p < 0.01$ ), suggesting that the ginsenoside augmented the *in vitro* cytotoxicity of curcumin. Viability of HCT116 was  $9.71 \pm 5.35\%$  and  $0.60 \pm 2.61\%$  at 10 and  $20 \mu\text{g/mL}$  of curcumin. Likewise, viability of HT29 cell line was  $35.17 \pm 4.11\%$  and  $10.75 \pm 5.79\%$  at 10 and  $20 \mu\text{g/mL}$  of



**Fig. 2.** *In vitro* cytotoxicity of colon cancer cell lines after treatment with curcumin or nanostructured lipid carriers loaded with curcumin. C-DMSO, curcumin dissolved in DMSO (stock solution, 10 mg/mL as curcumin); G-NLC, ginsenoside-modified nanostructured lipid carrier loaded with curcumin; NLC, nanostructured lipid carrier loaded with curcumin. \*\* $p$  value  $< 0.01$ ; ns, not significant. **a** HCT116 cell line; **b** HT29 cell line. Each value represents the mean  $\pm$  SD ( $n = 6$ ). Volume of the DMSO without curcumin, NLC without curcumin, and G-NLC without curcumin was used with the same of volume of the C-DMSO, NLC, and G-NLC for each concentration designated in the x-axis, respectively



**Fig. 3.** Fluorescence microscopic image of colon cancer cell lines after treatment with nanostructured lipid carriers loaded with curcumin. G-NLC, ginsenoside-modified nanostructured lipid carrier loaded with curcumin; NLC, nanostructured lipid carrier loaded with curcumin. **a** HCT116 cell line; **b** HT29 cell line

curcumin in G-NLC, respectively. Blank G-NLC (G-NLC without curcumin) was nontoxic up to nanoparticle concentration of 400  $\mu\text{g}/\text{mL}$  (equivalent to 20  $\mu\text{g}/\text{mL}$  of curcumin if loaded).

Figure 3 shows the intracellular fluorescence, conferred by the cellular uptake of curcumin and/or curcumin-loaded nanoparticles in HCT116 and HT29 cell lines. Both cell lines emitted green fluorescence, and the morphology of the cells was intact at treatment time of 30 min. Furthermore, the green fluorescence was spread homogeneously across all cells, indicating that curcumin had been taken up by the cells.

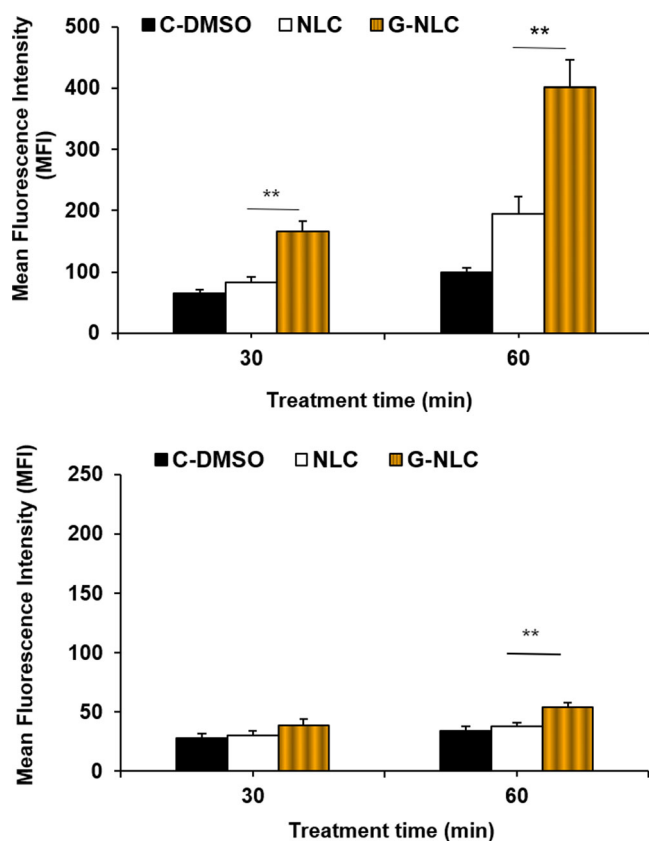
The fluorescence of HCT116 cells treated with G-NLC for 30 min was remarkably stronger than that of cells treated with C-DMSO or NLC (Fig. 4). Conversely, no significant difference was observed in HT29 cells. The intensity of fluorescence increased in both cell lines when the cells were treated for 60 min. The fluorescence intensity after of HCT116 cells increased 2.0-fold after 30- and 60-min treatments with G-NLC (5  $\mu\text{g}/\text{mL}$  as curcumin) compared with NLC-treated HCT116 cells. Uptake by HT29 cells increased 1.4-fold and, after 60-min treatment, no further significant increase in cytotoxicity of these cells was observed. These two findings indicate that curcumin and/or curcumin-loaded NLC is taken up by the cells. Furthermore, the incorporation of ginsenoside into the NLC significantly augmented the internalization of the nanoparticles into the colon cancer cell lines, especially in HCT116 cells.

Patients took G-NLC (100 mg curcumin twice daily), and blood samples were collected after 6 and 12 weeks to determine the concentration of curcumin and curcumin glucuronide in plasma. Unbound curcumin in plasma after 6 and 12 weeks was  $2.90 \pm 0.92$  and  $4.97 \pm 0.78$  ng/mL, respectively (Fig. 5). The glucuronide metabolite of curcumin was found at a concentration of  $240.60 \pm 80.96$  ng/mL and  $249.59 \pm 66.15$  ng/mL after 6 and 12 weeks, respectively. The mass spectra of the precursor and product ions of curcumin, curcumin glucuronide, and hesperetin are illustrated in Fig. 6. No adverse events were reported during the study.

## DISCUSSION

A number of studies have identified the *in vitro* cytotoxicity of curcumin in a variety of cancer cell lines (19–21). Unlike some cytotoxic agents, which are harmful to healthy cells and physiology, curcumin either displays a positive pharmacological effect or is simply excreted without affecting normal cellular and bodily functions (22,23). As a result, an increasing number of researchers have been exploring the potential of curcumin for the treatment of cancer patients.

Curcumin suffers from very poor water solubility, and consequently, it barely enters systemic circulation, giving it very low bioavailability. We have previously measured the solubility of curcumin by the shake-flask method and found

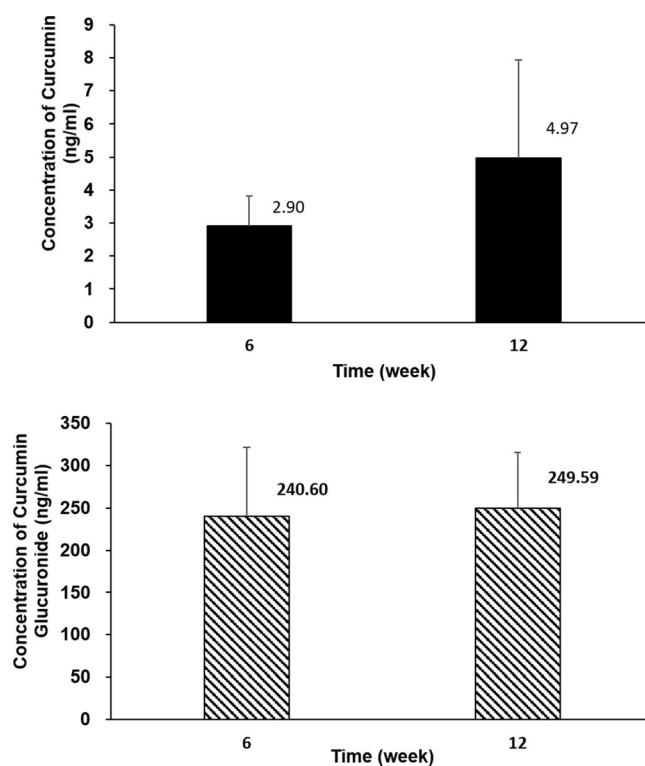


**Fig. 4.** Cellular uptake of curcumin in the colon cancer cell lines. C-DMSO, curcumin dissolved in DMSO; G-NLC, ginsenoside-modified nanostructured lipid carrier loaded with curcumin; NLC, nanostructured lipid carrier loaded with curcumin. **a** HCT116 cell line; **b** HT29 cell line. \*\* $p < 0.01$

that it was only  $2.7 \pm 0.2 \mu\text{g/mL}$  (17). Incorporating curcumin into a nanoparticle is one potential solution to address these limitations (9,24,25). Although many approaches have been investigated, only a few were able to exert even a minimum therapeutic effect in humans (8,26,27).

FACS analysis showed a higher uptake of curcumin when the NLC was modified with ginsenoside (G-NLC) compared to NLC prepared without ginsenoside in HCT116 cells (Figs. 3 and 4). Interestingly, HCT116 cells showed remarkably higher fluorescence intensity than HT29. Furthermore, HCT116 cells resulted in 2.0-fold increase of fluorescence intensity while it was only 1.4-fold in the HT29 cells (Fig. 4). Given that both HCT116 and HT29 cells belong to colon cancer but have different genotype, it appears that cellular uptake of curcumin may have different pathway for each cell's genotype. However, further study is warranted to elucidate mechanism for the increased cellular uptake of curcumin in HCT116 cells.

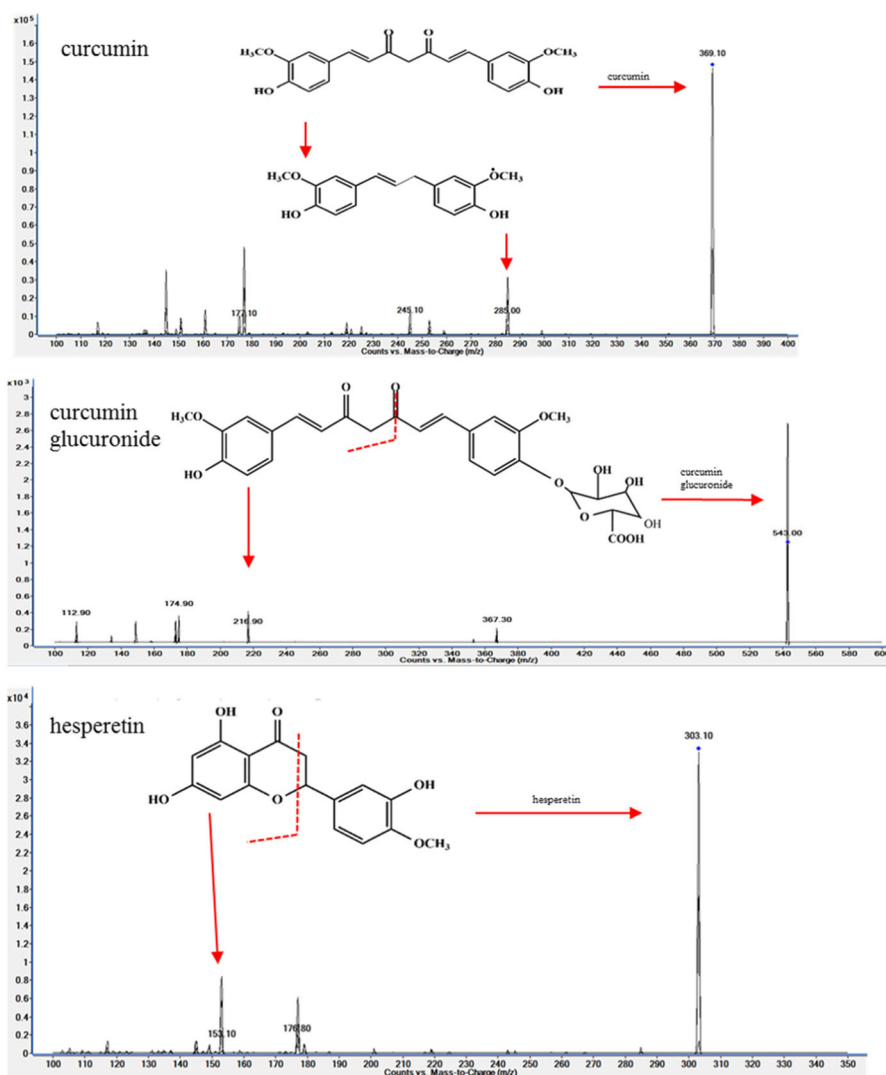
The *in vitro* cytotoxicity assay revealed that curcumin exhibited dose-dependent anticancer activity against the two colon cancer cell lines tested (Fig. 2). As shown in Figs. 2 and 4, the anticancer activity of curcumin is closely related with its cellular uptake of curcumin: the greater the uptake by the cell, the higher the anticancer activity. More specifically, curcumin was better taken up by HCT116 than by HT29 cells and, accordingly, the former were more susceptible to the anticancer activity of curcumin than the latter.



**Fig. 5.** Plasma level of curcumin and curcumin glucuronide in colon cancer patients ( $n = 10$ ). Dose: curcumin 100 mg was orally administered twice daily as G-NLC

Introduction of ginsenoside into NLC augmented its *in vitro* cytotoxicity in both cell lines. Interestingly, however, significant augmentation of cytotoxicity was also observed in HT29 at a curcumin concentration of  $10 \mu\text{g/mL}$  or higher. It appears that curcumin-containing NLC was well absorbed by HCT116 cells and, therefore, incremental activity of ginsenoside modification was not noticeable even at the highest concentration ( $20 \mu\text{g/mL}$ , Fig. 2a). On the other hand, in HT29 cells, which showed less cellular uptake of curcumin, the incremental activity began to appear significantly at  $10 \mu\text{g/mL}$  (Fig. 2b). This result suggests that ginsenoside modification of NLC may be a useful strategy for the treatment of colon cancer with similar genotype to HT29.

Earlier, we had reported that phospholipid-based NLC was effectively taken up by caveolae-mediated endocytosis pathway in colon cancer cells and the anticancer activity of oxaliplatin was significantly enhanced (28). In this study, we hypothesized that ginsenoside, a steroid glycoside, could exert beneficial effect on the bioavailability of curcumin because steroid-like molecule in the phospholipid-based delivery system may provide affinity to the system, facilitating endocytosis of the particles through the phospholipid bilayers embedded with cholesterol. However, in this study, we were not able to investigate the affinity of the delivery system to the colon cancer cell membrane. Another limitation of this study is that ginsenoside-modified NLC cannot be carried into tumors or blood stream. Therefore, G-NLC may never have the chance to enhance cellular uptake of curcumin in cancer cells. Further study is needed to develop an injectable formula so that G-NLC can be carried into blood circulation and get access directly to tumor cells.



**Fig. 6.** The mass spectra of precursor and product ions of curcumin, curcumin glucuronide, and hesperetin

We reported that the AUC of unbound curcumin was  $18.47 \pm 6.87$  and  $74.09 \pm 14.26$  h  $\times$  ng/mL ( $p < 0.01$ ) in rats at doses of 50 and 100 mg/kg of curcumin (administered as G-NLC), respectively, while unbound curcumin was not even detected when the same amount of curcumin was administered as pure powder (17). In the present, administration of G-NLC in colon cancer patients produced appreciable plasma levels of unbound curcumin (the compound responsible for antioxidant activity) at a comparable dose to that used by Cuomo and his colleagues (29). In a clinical study involving nine healthy subjects, the authors reported that unbound curcumin could not be detected in any plasma sample, so they treated the sample with glucuronidase to generate unbound curcumin from the conjugate. They achieved plasma level of 35.6 ng/mL curcumin glucuronide, which is equivalent to 24.2 ng/mL as curcumin, at comparable dosage to our study protocol. Compared to their results, plasma level of curcumin glucuronide we achieved is as high as seven times. This result represents significant progress in improving the bioavailability of curcumin. G-NLC was well tolerated by all subjects for the entire 12-week duration of our study.

Patients reported good oral palatability when consuming G-NLC dispersion, and no untoward effects were reported.

## CONCLUSIONS

Introduction of ginsenoside into the coating of curcumin-loaded NLC increased its uptake by colon cancer cells. In the HCT116 cell line, cellular uptake was increased 2.0-fold by ginsenoside modification, but it did not result in significant increase in the anticancer activity of curcumin. In contrast, cellular uptake by the HT29 cell line increased by a smaller amount, but significant incremental anticancer activity was observed beginning at 10  $\mu$ g/mL. Our study suggests that ginsenoside modification of curcumin-loaded NLC may be a useful strategy in the treatment of colon cancer with similar genotype to HT29.

## ACKNOWLEDGEMENTS

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## FUNDING INFORMATION

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## COMPLIANCE WITH ETHICAL STANDARDS

The study protocol was approved by the Institutional Review Board of Gachon University Gil Medical Center (GAIRB2015-87), and written informed consent was obtained from patients before their entry into the study.

**Conflict of Interest** The authors declare that they have no conflict of interest.

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