보건학석사 학위논문

Selective Effects of Curcumin on CdSe/ZnS Quantum Dots induced Phototoxicity Using UVA Irradiation in Normal Human Lymphocytes and HL-60 cells

Curcumin이 정상세포와 암세포에서 **UVA**에 의한
CdSe/ZnS Quantum Dots의 선택적 광독성에
미치는 영향 연구

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Abstract

Selective Effects of Curcumin on CdSe/ZnS Quantum Dots induced Phototoxicity Using UVA Irradiation in Normal Human Lymphocytes and HL-60 cells

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Quantum dots have received considerable attention due to their potential role in photosensitization during photodynamic therapy. Although Quantum dots are attractive nanomaterials due to their novel and unique physicochemical properties, concerns about their toxicity remain. This study suggests a combination strategy, CdSe/ZnS Quantum dots together

with curcumin, a natural yellow pigment from turmeric, to reduce Quantum dot-induced cytotoxicity.

The aim of this study was to explore a potentially effective cancer treatment: co-exposure of HL-60 cells and human normal lymphocytes to CdSe/ZnS Quantum dots and curcumin. Cell viability, apoptosis, reactive oxygen species (ROS) generation, and DNA damage induced by CdSe/ZnS Quantum dots and/or curcumin with or without ultraviolet A (UVA) irradiation were evaluated in both HL-60 cells and normal lymphocytes.

In HL-60 cells, cell death, apoptosis, ROS generation, and single/double DNA strand breaks induced by CdSe/ZnS Quantum dots were enhanced by treatment with curcumin and UVA irradiation. The protective effects of curcumin on cell viability, apoptosis, and ROS generation were observed in normal lymphocytes, but not leukemia cells.

These results demonstrated that treatment with CdSe/ZnS Quantum dots combined with curcumin increased cell death in HL-60 cells, which was mediated by ROS generation. However, curcumin acted as an antioxidant in cultured human normal lymphocytes.

Keywords: Curcumin, CdSe/ZnS Quantum dots, Photodynamic therapy, Phototoxicity, Reactive oxygen species, Apoptosis, DNA damage

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I. Introduction

Nanomedicine, integrated nanotechnology and medicine, is used to improve human health and has become one of the most promising and attractive nanotechnology fields (Hauck *et al.*, 2010; Choi *et al.*, 2012). Quantum dots (QDs), semiconductor nanocrystals composed of a quantum dot core and a shell, have unique optical and physicochemical properties, such as wide absorption spectra, narrow emission bands, high photostability, and photoluminescence (Chibli *et al.*, 2011; Morosini *et al.*, 2011). Because of their novel characteristics, Quantum dots have become very attractive nanomaterials for bioimaging and cancer therapy in nanomedicine (Choi *et al.*, 2012). However, cadmium-containing Quantum dots (CdSe Quantum dots) have raised concerns regarding toxicity. Although the toxicity of CdSe-based Quantum dots can be somewhat reduced by incorporating a protective zinc-sulfide (ZnS) inorganic shell, the release of Cd^{2+} ions eventually leads to toxic effects on biological systems (Pons *et al.*, 2010). Furthermore, it was recently reported that the cytotoxicity of CdTe/CdS (core-shell) and CdTe/CdS/ZnS (core-shell-shell) Quantum dots is also due to intracellular distribution of Quantum dots in cells and the associated nanoscale effects (Valizadeh *et al.*, 2012).

Previous study showed that CdSe core/ZnS shell Quantum dots induced cytotoxicity and genotoxicity in human normal lymphocytes as well as human lung cancer cells. Specifically, Quantum dots phototoxicity was remarkably increased following UV irradiation via reactive oxygen species (ROS) generation, single- and double-stranded DNA damage, and finally, apoptotic and/or necrotic cell death, which implies potential photodynamic therapy (PDT) applications for lung cancer cells (Choi *et al.*, 2012). Numerous studies have strived to reduce Quantum dots toxicity and render them water-soluble for biological applications; potential methods of reducing toxicity include synthesis of Quantum dot-gelatin nanocomposites,

cadmium-free Quantum dots, and biofunctionalized (polymer or peptide conjugates) Quantum dots (Byrne *et al.*, 2007; Mazumder *et al.*, 2009; Pons *et al.*, 2010).

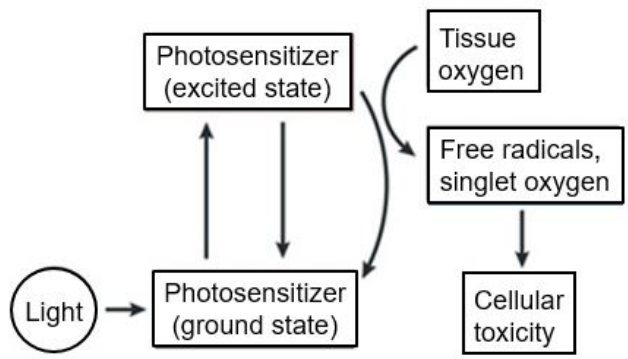

Figure 1 Mechanism of action of photodynamic therapy (PDT)

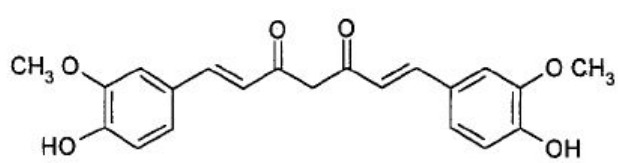

Figure 2 Chemical structure of curcumin

Curcumin, a natural yellow pigment isolated from turmeric (*Curcuma longa Linn*), is an anti-inflammatory, antioxidant, antimicrobial, and anticancer agent, and has long been used as a food addictive and medicinal agent (Ayli *et al.*, 2010; Dovigo *et al.*, 2011; Zhang *et al.*, 2012). Curcumin exerts dual actions, both as an antioxidant and cytotoxicant (Huang *et al.*, 2011). In addition to its cytotoxic anticancer effects, curcumin reduces oxygen free radicals, prevents lipid peroxidation, and attenuates DNA damage (Zhang *et al.*, 2012). Curcumin has been investigated extensively in human melanoma, and head and neck, breast, colon, pancreatic, prostate and ovarian cancers. Curcumin exerts its anticancer effects by stimulating apoptosis, regulating cellular growth, suppressing nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, modulating autophagy, and inhibiting tumor angiogenesis (Wilken *et al.*, 2011). Among these mechanisms, curcumin-induced apoptosis is the main pathway targeted in anticancer strategies. Apoptosis is targeted in various ways, including decreasing the expression of antiapoptotic members of the Bcl-2 family and increasing the expression of proapoptotic members (*e.g.*, Bax, caspase-3, -8, and -9) (Ahn *et al.*, 2012). Curcumin, which possesses photosensitizing and phytochemical properties and has chemopreventive potential, has gained attention for use in developing adjuvant chemotherapies or PDT to increase therapeutic efficacy and reduce side effects (Park and Lee, 2007; Wilken *et al.*, 2011; Ahn *et al.*, 2012). However, the cytotoxic and preventive effect of curcumin against nanoparticle-induced toxicity on cancer cells and normal cells has not been reported.

Effective cancer treatment protocols that combine photoactive nanoparticles and natural products without damaging normal cells are of great interest. In this study, the effect of a combination of CdSe/ZnS Quantum dots and curcumin under UVA irradiation on apoptotic cell death in HL-60 cells and the protective effect of curcumin against CdSe/ZnS Quantum dot-induced cytotoxicity and genotoxicity in human normal lymphocytes were examined.

II. Materials and methods

1. Cell culture

A human promyelocytic leukemia cell line (HL-60) was obtained from the Korean Cell Line Bank (Seoul, Korea) and cultured in RPMI 1640 medium supplemented with filtered 10% fetal bovine serum (FBS), penicillin and streptomycin (100 ㎍/㎖ of each) at 37ºC in a humidified atmosphere of 5% CO_2. CdSe/ZnS Quantum dots (Lumidot™) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Freshly drawn, heparinized blood from a donor was used to isolate lymphocytes by Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden) density gradient centrifugation at 400 × g. After extraction from the gradient interface, lymphocytes were washed twice with phosphate buffered saline (PBS) and resuspended in RPMI 1640 medium (Gibco, Invitrogen, Carlsbad, CA, USA) containing 10% FBS and 100 ㎍/㎖ each of penicillin and streptomycin. Lymphocytes were stimulated with 1% phytohemagglutinin (PHA; Gibco, Invitrogen, CA, USA) and cultured in a humidified atmosphere at 37ºC and 5% CO_2.

2. Materials

2.1 Preparation and characterization of CdSe/ZnS Quantum dots

CdSe/ZnS Quantum dots dispersed in toluene were supplied by Sigma-Aldrich (St. Louis, MO, USA). The same CdSe/ZnS Quantum dot nanoparticles were used (Choi *et al.*, 2012). Briefly, the toluene was removed using rotary evaporation (Rotavapor R-210; Büchi Laboratory Equipment, Zurich, Switzerland) and suspended the CdSe/ZnS Quantum dots in distilled water, which was sonicated for 30 minutes at 4ºC before each experiment. Dynamic light scattering (DLS), an indirect method of particle size determination, was measured at room temperature with an ALV/CGS-3 Compact Goniometer System (Hessen, Germany) equipped with a He-Ne laser operating at 632.8 ㎚. Transmission electron microscopy (TEM; 100CX; JEOL, Tokyo, Japan) was used to determine the size and shape of the CdSe/ZnS Quantum dots. Absorption peaks of the CdSe/ZnS Quantum dots were measured using an ultraviolet-visible (UV-Vis) spectrophotometer (V-650; Jasco, Tokyo, Japan), and photoluminescence spectra were detected with a fluorescence spectrophotometer (F4500; Hitachi, Tokyo, Japan). The CdSe/ZnS Quantum dot surface charge was measured in distilled water using a zeta potential analyzer (Zeta plus; Brookhaven Instruments Corp., Holtsville, NY, USA).

2.2 Curcumin preparation

Curcumin (Sigma-Aldrich, St. Louis, MO, USA) was diluted in dimethyl sulfoxide (DMSO) and added to cells at 0.5, 1, 2, and 4 ㎎/㎖. The final concentration of DMSO was 1%, which had no effect on cell viability (Figure 3).

3. Evaluation of cell viability

Cytotoxic effects induced by CdSe/ZnS Quantum dots and/or curcumin in HL-60 cells and normal human lymphocytes were evaluated by the Water Soluble Tetrazolium-1 (WST-1) assay (Daeil Lab Service Co. Ltd., Seoul, Korea). Two types of cells were seeded in each 96-well plate at a density of 1×10^6 cells/100 ㎕, pretreated with curcumin for 1 hour, and incubated for 24 hours at 37ºC in a humidified atmosphere of 5% CO_2. Sequentially, the cells were treated with CdSe/ZnS Quantum dots at various concentrations with/without UVA (2J/㎠) irradiation. A Bio-Link BLX irradiation system (Vilber-Lourmat, Marne-la-Vallee Cdex 1, France) equipped with five 8-W UV lamps (UVA Vilber-Lourmat T- 8L with peak irradiance at 365) was used. The WST-1 solution 100 ㎕ was added to each well and the plate was incubated for 4 hours. The absorbance at 492 ㎚ was measured using a microplate reader (Tecan, Männedorf, Switzerland).

4. Single-cell gel electrophoresis (comet assay)

Single-cell gel electrophoresis was performed as described by Singh (Singh *et al.*, 1988). HL-60 cells and human normal lymphocytes were treated with curcumin and/or CdSe/ZnS Quantum dots for 1 hour and 3 hours, respectively, followed by UVA (2 J/㎠) exposure. After two PBS washes, the cells were maintained at 4°C to prevent DNA repair. Slides were prepared according to the method of Singh (Singh *et al.*, 1988). Briefly, images of 70 cells randomly selected from each sample were analyzed using a Komet 5.5 image analysis system (Kinetic Imaging Ltd., Nottingham, UK). The Olive tail moment (OTM) of each cell was measured under a fluorescence microscope (Nikon, Tokyo, Japan) equipped with a 515 to 560 ㎚ excitation filter and 590 ㎚ barrier filter.

5. Cytokinesis-block micronucleus assay

The cytokinesis-block micronucleus (CBMN) assay was performed as described by Fenech (Fenech, 1993). HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% FBS, incubated with curcumin and/or CdSe/ZnS Quantum dots for 1 hour and 3 hours, respectively, and then irradiated with UVA. Cytochalasin B (4 ㎍/㎖; Sigma-Aldrich, St. Louis, MO, USA) was added 20 hours after the start of culture, and the incubation was continued for an additional 28 hours.

Human peripheral whole blood 1 ㎖ was cultured in 9 ㎖ of RPMI 1640 media supplemented with 10% FBS and treated with curcumin and/or CdSe/ZnS Quantum dots under the same conditions as the HL-60 cells. After 44 hours incubation, cytochalasin B was added to the culture and the cells were incubated for another 28 hours.

After a total incubation period of 48 hours for HL-60 cells and 72 hours for whole blood lymphocytes, the cells were harvested, treated with hypotonic 0.075 M KCl solution for 1 minute, and washed twice with fixative solution (methanol : acetic acid, 3 : 1). Air-dried cell preparations were stained with 5% Giemsa solution. In total, 1,000 binucleated cells with well-preserved cytoplasm were scored according to standard criteria (Fenech, 2000). In addition, CBPI (cytokinesis-block proliferation index) was evaluated in 500 cells from each sample in HL-60 cells and normal lymphocytes to determine the percentage of bi- and multi-nucleated cells.

6. Measurement of ROS

Intracellular ROS generation was assayed using 5-(and-6)-carboxy-2', 7'-dichlorofluorescein diacetate (DCFDA; Molecular Probes, Eugene, OR, USA). HL-60 cells and isolated lymphocytes were treated with curcumin for 1 hour and CdSe/ZnS Quantum dots for 3 hours, and then irradiated with UVA. Thereafter the medium was discarded through centrifugation, and fresh culture medium containing 20 μM DCFDA was added in the dark. The cells were incubated for 20 minutes at 37ºC and fluorescence distribution was monitored by flow cytometry (FACS Caliber, Becton-Dickinson, CA, USA).

7. Measurement of apoptosis

Apoptosis induction by curcumin and/or CdSe/ZnS Quantum dots with/without UVA irradiation was determined by propidium iodide (PI) staining and flow cytometry. HL-60 cells and normal lymphocytes exposed to curcumin and/or CdSe/ZnS Quantum dots were collected, washed twice with PBS, and fixed with 70% cold aqueous ethanol for 24 hours and then stored at -20ºC for at least 24 hours. Cells were washed twice with PBS and cell pellets were stained with PI solution containing RNase A 10 ㎎/㎖ and PI 10 ㎎/㎖ in PBS. The cell suspension was incubated in the dark at room temperature for 30 minutes, and DNA content was assayed by flow cytometry (FACS Caliber, Becton-Dickinson, CA, USA).

8. Statistical analysis

The effects of curcumin and/or CdSe/ZnS Quantum dots with or without UVA irradiation on DNA were evaluated using non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) and the Mann-Whitney *U* test. One-way ANOVA and Student's *t*-tests were applied to analyze cell viability. A difference of $p < 0.05$ was considered to indicate statistical significance. Data are shown as means ± standard deviation (S.D.).

III. Results

1. Physicochemical characterization of CdSe/ZnS Quantum dots

In previous study, the average size of core-shell type CdSe/ZnS Quantum dots as determined by DLS (particle size, 3.3 ㎚; λ_{em}, 530 ㎚) was 105 ± 15.55 ㎚, indicating that Quantum dot particles aggregate in an aqueous environment (Table 1).

The CdSe/ZnS Quantum dot UV-Vis absorption spectrum showed broad continuous absorption at wavelengths ranging from UV to visible. The zeta potential measurement in distilled water showed a ζ potential of + 40.58 ± 7.97 ㎷, confirming the colloidal stability of CdSe/ZnS Quantum dots (Choi *et al.*, 2012).

Table 1 Average sizes of CdSe/ZnS Quantum dots in RPMI 1640 medium as determined by DLS.

Aggregate size comparisons for CdSe/ZnS Quantum dots after 0 and 24 hours.

Time (hours)	DLS (nm)
0	105 ± 15.55
24	125 ± 4.24

2. Effect of curcumin on HL-60 cell viability following Quantum dot treatment under UVA irradiation

WST-1 assays were performed in HL-60 cells to assess the cytotoxicity of curcumin and/or CdSe/ZnS Quantum dots with/without UVA irradiation. HL-60 cells were preincubated with curcumin for 1 hour and then treated with Quantum dots, followed by irradiation with UVA light. An overall concentration-dependent decrease in cell viability was observed in cells treated with Quantum dots and/or curcumin. The effect of curcumin as a photosensitizer was more significant than that of CdSe/ZnS Quantum dots. Significant reductions in cell viability of 60%, 50%, 35%, and 29% were observed for curcumin treatments of 0.5, 1, 1.5, and 2 ㎍/㎖, respectively, under UVA irradiation as compared with the control group, including the DMSO-treated negative control (Figure 3A). In CdSe/ZnS Quantum dot-treated cells, 88%, 85%, 65%, and 29% viabilities were observed following treatment with Quantum dot concentrations of 3, 5, 10, and 20 ㎍/㎖, respectively. Thus, combination treatment with CdSe/ZnS Quantum dots 5 ㎍/㎖ and curcumin 1 ㎍/㎖ under UVA irradiation was highly effective at killing HL-60 cancer cells (Figure 3B).

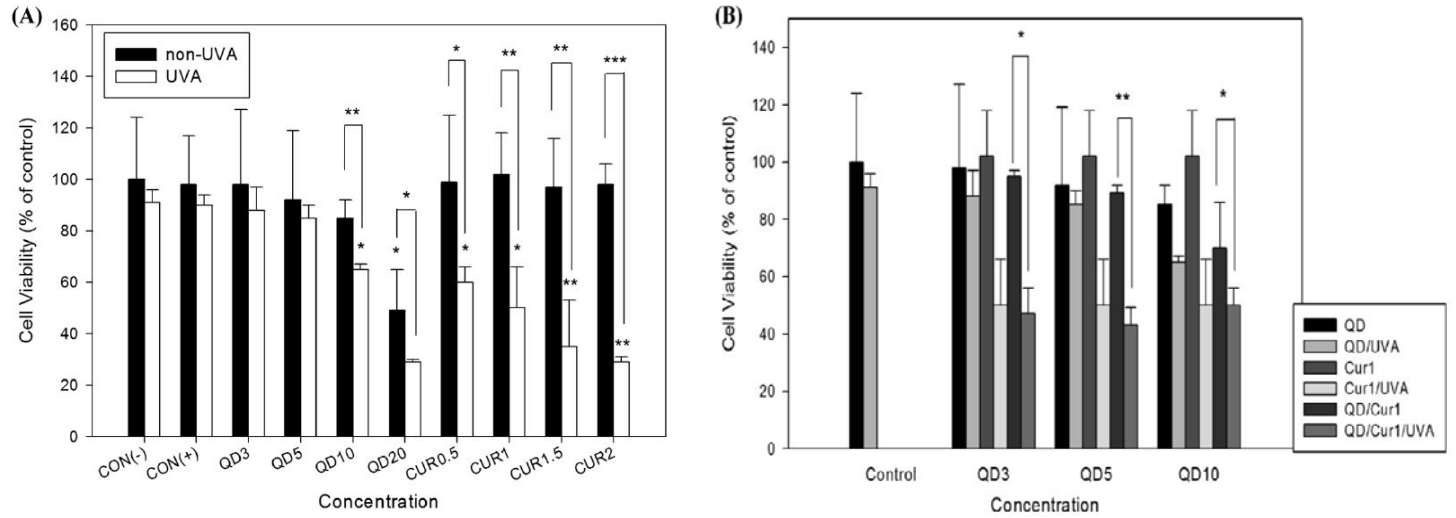

Figure 3 HL-60 cell viability following treatment with CdSe/ZnS Quantum dots and/or curcumin in the presence or absence of UVA irradiation. Cells were incubated in a 96-well microplate for 24 hours, treated with 3, 5, 10 or 20 ㎍/㎖ CdSe/ZnS Quantum dots or 0.5, 1, 1.5, or 2 ㎍/㎖ curcumin, respectively (A) and co-exposed to 3, 5, or 10 ㎍/㎖ CdSe/ZnS Quantum dots and 1 ㎍/㎖ curcumin (B). Untreated control, CON(-); negative control (treated with DMSO), CON(+). Cell viability was determined by the WST-1 assay. Data are shown as percentages of the control, and error bars represent standard deviations (S.D.) of duplicate experiments. Results were evaluated statistically by one-way ANOVA and Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Effect of curcumin on normal lymphocyte viability following Quantum dot treatment under UVA irradiation

To assess the effect of curcumin on Quantum dot-induced cytotoxicity in normal lymphocytes isolated from whole blood, the WST-1 assay was used. CdSe/ZnS Quantum dot treatment decreased the viability of normal lymphocytes in a concentration-dependent manner, but curcumin with/without UVA irradiation had a similar effect on normal lymphocytes as in control groups (Figure 4A). Curcumin treatment protected against CdSe/ZnS Quantum dot-induced cytotoxicity in normal lymphocytes, as seen in Figure 4B.

CdSe/ZnS Quantum dots 5 and 10 ㎍/㎖ in combination with 1 ㎍/㎖ curcumin increased cell viabilities by 90% and 73% respectively, which were reduced by UVA irradiation to 79% and 55%, as compared with CdSe/ZnS Quantum dots alone (68% and 46%, respectively). Although UVA irradiation decreased cell viability somewhat, the protective effect of curcumin on normal lymphocytes was statistically significant ($p < 0.05$).

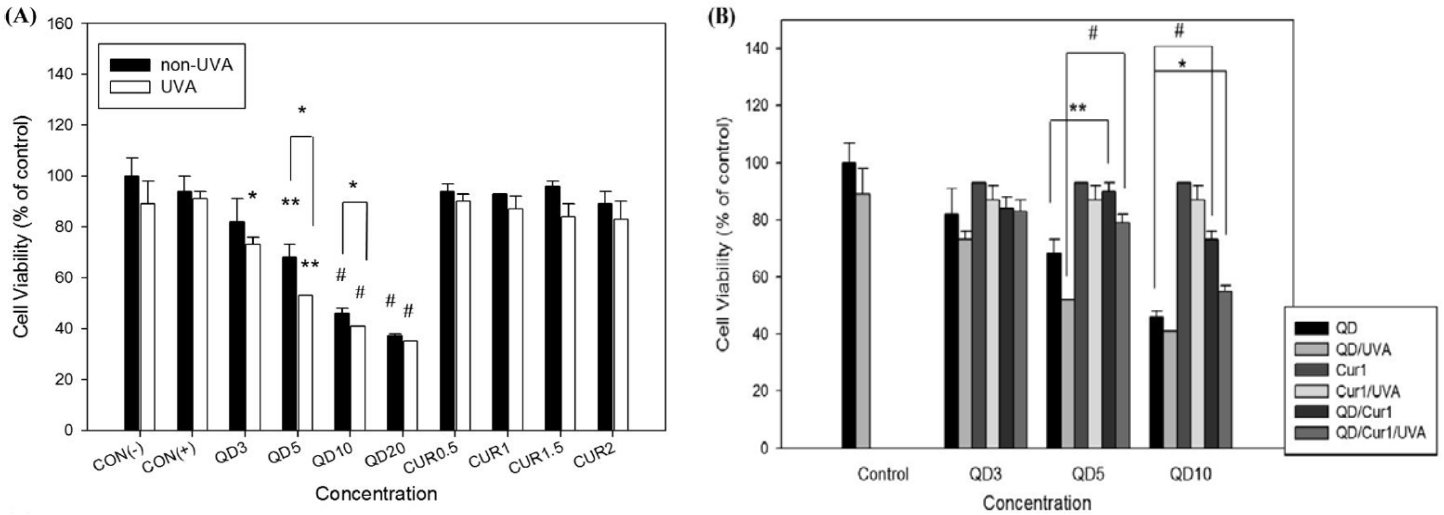

Figure 4 Cultured human normal lymphocyte cell viability following treatment with CdSe/ZnS Quantum dots and/or curcumin in the presence or absence of UVA irradiation. Cells were incubated in a 96-well microplate for 24 hours, treated with 3, 5, 10, 20 ㎍/㎖ CdSe/ZnS Quantum dots or 0.5, 1, 1.5, 2 ㎍/㎖ curcumin, respectively (A) and co-exposed to 3, 5, 10 ㎍/㎖ CdSe/ZnS Quantum dots and 1 ㎍/㎖ curcumin (B). CON(-), untreated control; CON(+), negative control (treated with DMSO). Cell viability was determined by the WST-1 assay. Data are shown as percentages of the control and error bars represent standard deviations of duplicate experiments. Results were assessed statistically by one-way ANOVA and Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Effect of curcumin on CdSe/ZnS Quantum dot-induced DNA damage as indicated by the comet assay

The comet assay was evaluated genotoxicity induced by curcumin and/or CdSe/ZnS Quantum dots with/without UVA irradiation in HL-60 cells and normal human lymphocytes, in which the extent of DNA damage is represented by OTM (Figure 5). Significant DNA damage was evident in HL-60 cells treated with 10 ㎍/㎖ CdSe/ZnS Quantum dots plus UVA irradiation (OTM: 19.45 ± 1.05), cells treated with 3 ㎍/㎖ CdSe/ZnS Quantum dots and curcumin (OTM: 18.62 ± 2.11), and cells treated with 3 ㎍/㎖ CdSe/ZnS Quantum dots and curcumin plus UVA (OTM 18.59 ± 1.02) as compared with the control group. Combination treatment with curcumin and CdSe/ZnS Quantum dots induced statistically significant single-stranded DNA damage in HL-60 leukemia cells (Figure 5A).

OTM in normal lymphocytes treated with 3 ㎍/㎖ CdSe/ZnS Quantum dots and 1 ㎍/㎖ curcumin was slightly increased as compared with the control group (OTM: 11.2 ± 0.72 for 3 ㎍/㎖ CdSe/ZnS Quantum dots plus curcumin and 9.6 ± 0.51 for control). In this study, no protective effect of curcumin against CdSe/ZnS Quantum dot-induced DNA single strand breaks was found in normal lymphocytes (Figure 5B).

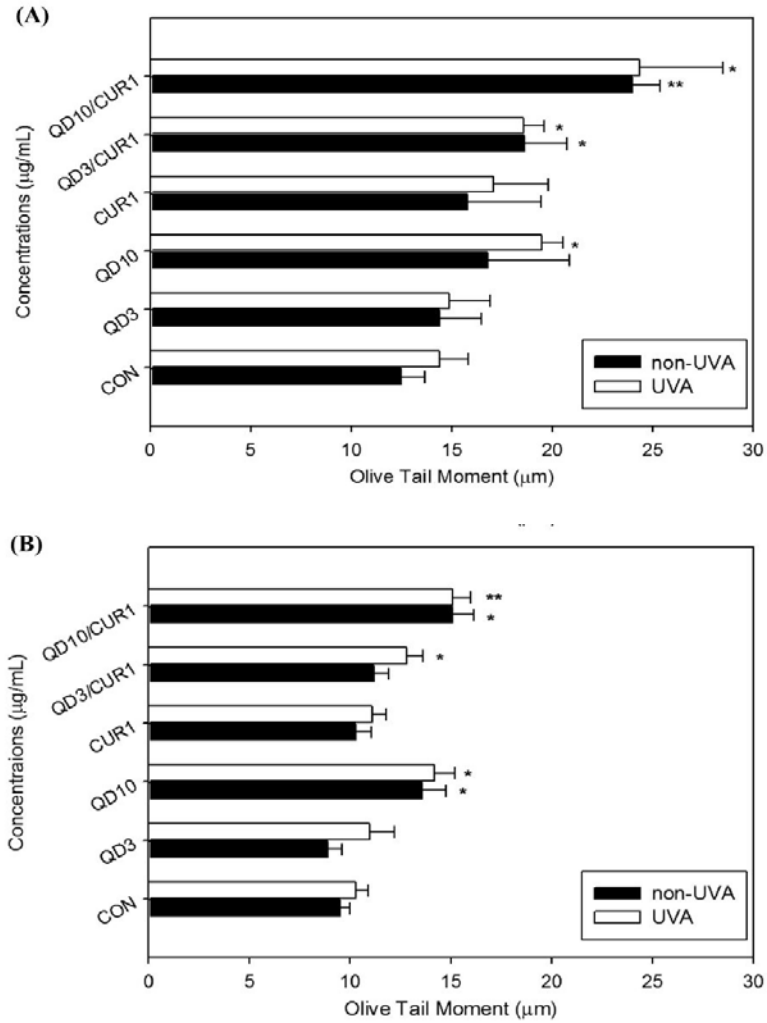

Figure 5 Single-stranded DNA breaks in HL-60 cells treated with CdSe/ZnS Quantum dots and/or curcumin with/without UVA irradiation. Single-stranded DNA breakage was evaluated by Olive tail movements (OTM) using the comet assay in HL-60 cells (A), and human normal lymphocytes (B). Values represent means ± standard deviation (S.D.). Results were analyzed statistically by Kruskal-Wallis and Mann-Whitney's *U* tests. * $p < 0.05$, ** $p < 0.01$ compared to the control

5. Effect of curcumin on CdSe/ZnS Quantum dot-induced DNA damage by micronucleus assay

MN assays also performed to assess the genotoxic effects of curcumin on CdSe/ZnS Quantum dots with/without UVA irradiation (Table 2). The MN frequency in HL-60 control cells was 2.5 ± 0.7 per 1,000 binucleated cells; the frequencies in cells treated with CdSe/ZnS Quantum dots at 3 and 10 ㎍/㎖ were 8.0 ± 1.4 and 17.0 ± 7.0, respectively. The frequencies of MN formation were 3.5 ± 0.7, 12.5 ± 2.1, and 19.5 ± 9.0 per 1,000 binucleated cells for control, 3, and 10 ㎍/㎖ CdSe/ZnS Quantum dots, respectively, with UVA irradiation. The MN frequencies induced by combination treatment with curcumin and CdSe/ZnS Quantum dots were 19.5 ± 9.0 and 31.5 ± 17.7 for 3 ㎍/㎖ CdSe/ZnS Quantum dots and 1 ㎍/㎖ curcumin, and 10 ㎍/㎖ CdSe/ZnS Quantum dots and 1 ㎍/㎖ curcumin, respectively. HL-60 cell MN frequencies were increased markedly when UVA irradiation was combined with CdSe/ZnS Quantum dots and curcumin treatment. MN frequencies induced by combination treatment with 3 ㎍/㎖ CdSe/ZnS Quantum dots or 10 ㎍/㎖ CdSe/ZnS Quantum dots and 1 ㎍/㎖ curcumin plus UVA irradiation were 24 ± 8.0 and 42.5 ± 9.2, respectively (Table 2). The reduction in CdSe/ZnS Quantum dot-induced MN formation in normal human lymphocytes with curcumin (reduction from 24.0 ± 5.6 to 22.0 ± 6.3) indicates that curcumin was more efficacious in protecting against genotoxicity, although not significantly so (Table 2). Finally, the combination treatment with curcumin and CdSe/ZnS Quantum dots increased MNs formation and decreased CBPI of cells.

Table 2 Induction of micronuclei (MN) in HL-60 cells and human normal lymphocytes treated with CdSe/ZnS Quantum dots and/or curcumin with/without UVA irradiation

HL-60 cells			Human normal lymphocytes		
Concentration (μg/mL)	Frequency of MNs/1000BNCs	CBPI	Concentration (μg/mL)	Frequency of MNs/1000BNCs	CBPI
Control	2.5 ± 0.7	1.63	Control	8.0 ± 3.0	1.39
Control/UVA	3.5 ± 0.7	1.50	Control/UVA	8.0 ± 1.0	1.31
QD3	8.0 ± 1.4*	1.58	QD3	17.0 ± 2.0*	1.32
QD3/UVA	12.5 ± 2.1*	1.49	QD3/UVA	23.0 ± 6.0*	1.26
QD10	17.0 ± 7.0	1.58	QD10	24.0 ± 6.0*	1.21
QD10/UVA	19.5 ± 9.0	1.39	QD10/UVA	28.0 ± 4.0*	1.17
Cur1	12.0 ± 8.5	1.68	Cur1	14.0 ± 6.0	1.31
Cur1/UVA	18.5 ± 7.8	1.44	Cur1/UVA	18.0 ± 4.0*	1.26
QD3/Cur1	19.5 ± 9.0	1.49	QD3/Cur1	22.0 ± 4.0*	1.31
QD3/Cur1/UVA	24.0 ± 8.0*	1.38	QD3/Cur1/UVA	22.0 ± 4.0*	1.30
QD10/Cur1	31.5 ± 17.7	1.47	QD10/Cur1	22.0 ± 6.0	1.30
QD10/Cur1/UVA	42.5 ± 9.2*	1.42	QD10/Cur1/UVA	26.0 ± 7.0*	1.24

Micronuclei induction was measured by cytokinesis-block micronucleus assay. MN, micronuclei; BNCs, binucleated cells; CBPI, cytokinesis-block proliferation index. Values represent means ± standard deviation (S.D.). Data were analyzed statistically using Kruskal-Wallis tests and Mann-Whitney *U*-tests.

* $p < 0.05$ compared to the control.

6. Effect of curcumin on CdSe/ZnS Quantum dot-induced intracellular ROS generation

ROS generation was determined by DCF fluorescence in HL-60 cells and detected by flow cytometry. Intracellular ROS content increased slightly in control cells treated with UVA, CdSe/ZnS Quantum dots treatment alone, CdSe/ZnS Quantum dots treatment with UVA exposure, and CdSe/ZnS Quantum dots plus curcumin co-treatment; ROS formation significantly increased in cells irradiated with UVA in combination with CdSe/ZnS Quantum dots and curcumin co-treatment. ROS generation in cells treated with 10 ㎍/㎖ CdSe/ZnS Quantum dots and curcumin plus UVA irradiation was increased four-fold compared to the control group (Figure 6). These results indicate that UVA irradiation enhances ROS generation in HL-60 cells treated with a combination of CdSe/ZnS Quantum dots and curcumin.

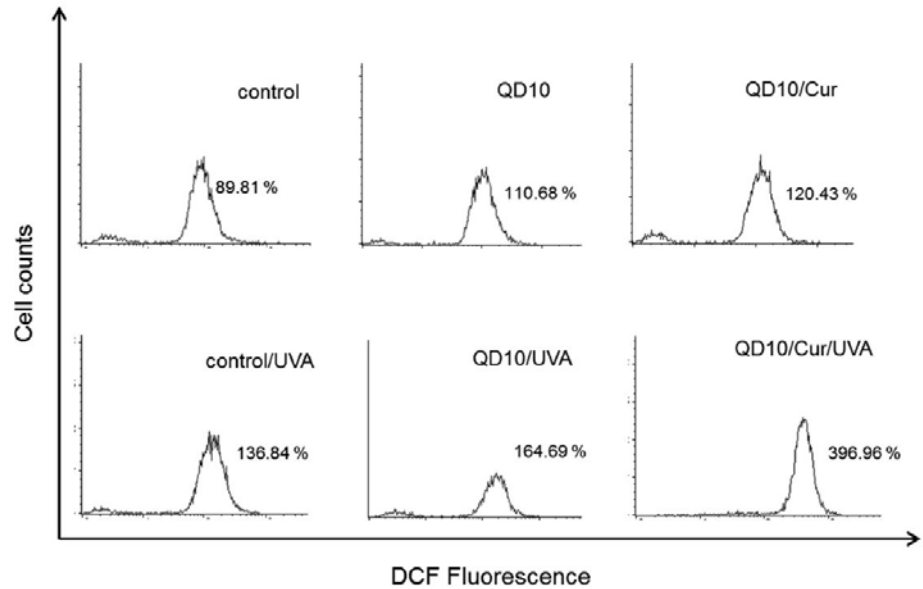

Figure 6 Effect of curcumin on reactive oxygen species (ROS) generation in CdSe/ZnS Quantum dot-treated HL-60 cells under UVA irradiation. HL-60 cells were pretreated with curcumin for 1 hour, prior to treatment with CdSe/ZnS Quantum dots. After 3 hours, the cells were irradiated with UVA light (2 J/㎠) and ROS generation was assayed by flow cytometry. Significant differences were observed following treatment with 10 ㎍/㎖ CdSe/ZnS Quantum dots and 1 ㎍/㎖ curcumin plus UVA irradiation as compared with the other treatment groups.

7. Assessment of apoptosis

The apoptosis assay was conducted to investigate the mode of CdSe/ZnS Quantum dots and/or curcumin-induced cell death in the presence or absence of UVA irradiation. HL-60 cells treated with CdSe/ZnS Quantum dots alone and/or exposed to UVA, co-treatment of CdSe/ZnS Quantum dots with curcumin and/or UVA exposure, and co-treatment of CdSe/ZnS Quantum dots with curcumin and/or UVA exposure, and co-treatment of CdSe/ZnS Quantum dots with curcumin and/or UVA exposure for 24 hours resulted in a concentration-dependent increase in apoptotic cell death, which increased to ~ 32.73% following CdSe/ZnS Quantum dots and curcumin treatment plus UVA irradiation, as compared to 6.98% for the untreated control. Apoptotic cell death occurred in ~ 16.97% of normal human lymphocytes following treatment with the same concentrations as in the HL-60 cells, as compared to 11.88% in the untreated controls (Table 3). These results clearly indicate that the CdSe/ZnS Quantum dots and curcumin combined treatment enhances apoptosis in cancer cells, whereas the combination has a protective effect in normal human lymphocytes.

Table 3 The percentages of apoptotic cells measured using flow cytometry in HL-60 cells and normal lymphocytes

Concentrations (μg/mL)	HL-60 cells	Normal lymphocytes
Control	6.98 ± 6.75	11.88 ± 7.77
QD5	7.21 ± 3.09	14.66 ± 11.81
QD10	8.05 ± 2.10	18.27 ± 9.44
Control/UVA	7.96 ± 6.26	13.81 ± 9.90
QD5/UVA	8.76 ± 7.26	15.99 ± 11.53
QD10/UVA	14.62 ± 3.10	18.72 ± 15.66
Cur1	5.74 ± 4.00	13.79 ± 5.15
QD5/Cur1	7.01 ± 2.30	9.92 ± 5.95
QD10/Cur1	13.04 ± 8.99	11.02 ± 5.94
Cur1/UVA	14.52 ± 13.34	15.39 ± 8.53
QD5/Cur1/UVA	22.60 ± 19.71	15.95 ± 9.55
QD10/Cur1/UVA	32.73 ± 23.20	16.97 ± 11.38

Data represent the mean ± S.D. obtained from two independent experiments. Results are statistically analyzed by one-way ANOVA test, but not significant in either group ($p > 0.05$)

IV. Discussion

Semiconductor nanocrystal Quantum dots, which display unique optical and electrical properties, are being used in many biological applications. Although Quantum dots have numerous novel physicochemical characteristics that make them one of the most advantageous tools in nanomedicine, many concerns as to human health have been raised due to their toxicity (Martin J. D. Clift, 2012). In a previous study established that CdSe/ZnS Quantum dots induced cytotoxicity and genotoxicity through DNA damage, ROS induction, and activation of the apoptotic signaling pathway in A549 cells; this study also proposed that they were a potential photosensitizer in photodynamic therapy (Choi *et al.*, 2012). The limitation of Quantum dot use due to their toxicity requires more effective cancer treatments that are selectively targeted toward cancer cells without damaging healthy tissue. Here, this study used a combined CdSe/ZnS Quantum dots and curcumin treatment, two potential photosensitizers, to destroy cancer cells and protect normal cells. Curcumin, a natural compound, is widely known as an antioxidant, anticlastogen, anti-inflammatory and anti-mutagenic agent that protects normal tissues (Zhang *et al.*, 2012). The aim of this study was to identify a more effective cancer treatment that does not damage normal cells by evaluating the selective effect of CdSe/ZnS Quantum dots and curcumin on HL-60 cells and normal human lymphocytes.

Upon comparing cell viability and apoptosis profiles between HL-60 cells and normal human lymphocytes, this study observed distinct sensitivities to the CdSe/ZnS Quantum dots and curcumin combined treatment, indicating the selective role of curcumin. Cell death and apoptosis were enhanced by curcumin combined with CdSe/ZnS Quantum dots plus UVA exposure in HL-60 cells (Figure 3 and Table 3). Unlike HL-60 cells, curcumin had a protective effect against CdSe/ZnS Quantum dot-induced toxicity on normal lymphocytes (Figure 4 and

Table 3). The selective effect of curcumin on the two cell types was also observed for ROS generation. ROS generation was markedly higher in HL-60 cells following co-treatment of CdSe/ZnS Quantum dots and curcumin with UVA exposure (Figure 6), while curcumin attenuated the CdSe/ZnS Quantum dot-induced ROS in normal lymphocytes (data not shown). This is consistent with previous reports that curcumin has antioxidant activity but acts as a pro-oxidant under certain conditions (Ahsan and Hadi, 1998; Mahakunakorn *et al.*, 2003; Cao *et al.*, 2006). ROS caused by CdSe/ZnS Quantum dots and curcumin exposure under UVA irradiation clearly increased single- and double-stranded DNA breaks as compared to CdSe/ZnS Quantum dots treatment alone, CdSe/ZnS Quantum dots and UVA co-exposure, curcumin treatment alone, curcumin and UVA co-exposure, or CdSe/ZnS Quantum dots and curcumin co-treatment in HL-60 cells (Figure 5A and Table 2). Although the protective effect of curcumin was not statistically significant, the micronuclei frequency was lower in CdSe/ZnS Quantum dots and curcumin-co-treated than in CdSe/ZnS Quantum dot-treated cultured normal lymphocytes.

This study results suggest that curcumin did not produce cytotoxicity and genotoxicity, but protected normal lymphocytes from CdSe/ZnS Quantum dot-induced cell death, apoptosis, and ROS generation regardless of UVA irradiation. In contrast to the normal lymphocytes, curcumin enhanced apoptotic cell death, ROS generation, and DNA damage induced by CdSe/ZnS Quantum dots in HL-60 cancer cells. Furthermore, the reduced cell viability was precipitated by UVA irradiation with CdSe/ZnS Quantum dots and curcumin in leukemia cells. The effect of curcumin in inducing apoptosis in cancer cells as opposed to normal cells is dependent upon various mechanisms, such as down-regulation of cyclin D and G2-M phage arrest in the cell proliferation pathway, a reduction in glutathione (GSH) levels resulting from ROS generation, caspase pathway activation, and higher uptake of curcumin by cancer cells (Syng-Ai *et al.*, 2004; Choudhuri *et al.*, 2005; Kunwar *et al.*, 2008; Ravindran *et al.*, 2009).

Taken together, this study data suggests that CdSe/ZnS Quantum dots and curcumin co-

exposure with UVA irradiation dramatically increased apoptotic cell death in HL-60 cells, and that this effect was mediated by ROS generation. Conversely, curcumin acted as an antioxidant in normal lymphocytes. The results of the present study indicate a novel treatment strategy for leukemia using a combination of CdSe/ZnS Quantum dots and curcumin.

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국문초록

Curcumin이 정상세포와 암세포에서 UVA에 의한 CdSe/ZnS Quantum Dots의 선택적 광독성에 미치는 영향 연구

Quantum Dots는 자외선에서부터 가시광선까지의 넓은 흡수 스펙트럼과 광안정성 및 발광과 같은 독특한 물리 · 화학적 성질을 가지며, 크기에 따라 다양한 빛을 발생할 수 있어 각종 디스플레이 소자나 조명, 태양전지 등에 쓰이고 있다. 특히 광역학치료에 있어 잠재적 역할을 가진 광감작제로 나노 의학분야에서 널리 이용되고 있으나, 정상세포에 미치는 영향과 세포 및 유전 독성에 대한 자세한 기전 연구는 부족한 실정이다.

광화학적 특성과 암 예방 효과를 가지고 있는 폴리페놀 성분의 노란색 천연물질인 curcumin은 다양한 종류의 암세포에서 세포 사멸을 유도하고, 항종양, 항산화, 항균, 항염 작용 등의 다양한 특성을 지니고 있다. 따라서 광감작제로서의 효과를 확인하고, CdSe/ZnS Quantum Dots와 병용 처리를 통해 정상세포를 보호하고 암세포를 선택적으로 사멸시키는 효과적인 광역학치료의 가능성을 검토하고자 하였다.

본 연구에서는 인간 급수 백혈병 세포주인 HL-60 세포와 사람의 말초혈액에서 분리한 정상 림프구에 curcumin과 CdSe/ZnS Quantum Dots를 단독 및 병용 처리하고 UVA를 조사하였을 때, 암세포의 증식 억제와 apoptosis 유도 효과를 알아보고, 이러한 효과에 따른 세포 독성과 유전 독성을 알아보고자 WST-1 assay와 소

핵 분석, 세포 내 ROS 농도 측정, 세포 사멸 및 DNA 절단 가닥을 분석하였다.

Curcumin과 CdSe/ZnS Quantum Dots를 단독 및 병용 처리하여 UVA를 조사하였을 때 Leukemia cell line인 HL-60 세포의 사멸을 유도하고, 정상세포는 보호하는 효과를 보였다. 특히 CdSe/ZnS Quantum Dots과 curcumin을 병용 처리한 HL-60 세포는 농도 의존적으로 세포 생존율이 감소하였고, UVA를 조사하였을 때 그 손상 정도가 매우 유의하게 높았다.

또한, curcumin은 정상세포에서 세포 및 유전 독성을 발생시키지 않으며, CdSe/ZnS Quantum Dots과 UVA에 의한 세포 죽음과 세포 사멸, ROS 생성에 대하여 정상세포를 보호하는 효과를 보였다. 정상세포에서와는 달리, curcumin은 급성 백혈구 세포주인 HL-60 세포에서 CdSe/ZnS Quantum Dots와 UVA를 동시에 처리하였을 때 선택적인 암세포 사멸 효과를 확인함으로써, 광역학치료에 있어 보다 효율적이고 유용하게 활용될 수 있을 것으로 기대된다.

표제어: Curcumin, CdSe/ZnS Quantum dots, 광역학치료법, UVA, 광독성, ROS, 세포 사멸

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