



## Selective Effects of Curcumin on CdSe/ZnS Quantum-dot-induced Phototoxicity Using UVA Irradiation in Normal Human Lymphocytes and Leukemia Cells

Soomin Goo<sup>†</sup>, Young Joo Choi<sup>†</sup>, Younghyun Lee, Sunyeong Lee and Hai Won Chung

School of Public Health and Institute of Health and Environment, Seoul National University, Seoul, Korea

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Quantum dots (QDs) have received considerable attention due to their potential role in photosensitization during photodynamic therapy. Although QDs are attractive nanomaterials due to their novel and unique physicochemical properties, concerns about their toxicity remain. We suggest a combination strategy, CdSe/ZnS QDs together with curcumin, a natural yellow pigment from turmeric, to reduce QD-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 QDs and curcumin. Cell viability, apoptosis, reactive oxygen species (ROS) generation, and DNA damage induced by QDs 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 QDs 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 QD 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.

**Key words:** CdSe/ZnS QDs, Curcumin, Apoptosis, Reactive oxygen species, DNA damage

### INTRODUCTION

Nanomedicine, integrated nanotechnology and medicine, is used to improve human health and has become one of the most promising and attractive nanotechnology fields (1,2). 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 (3,4). Because of their novel characteristics, QDs have become very attractive nanomaterials for bioimaging and cancer therapy in nanomedicine (2). However,

cadmium-containing QDs (CdSe QDs) have raised concerns regarding toxicity. Although the toxicity of CdSe-based QDs can be somewhat reduced by incorporating a protective zinc-sulfide (ZnS) inorganic shell, the release of Cd<sup>2+</sup> ions eventually leads to toxic effects on biological systems (5). Furthermore, it was recently reported that the cytotoxicity of CdTe/CdS (core-shell) and CdTe/CdS/ZnS (core-shell-shell) QDs is also due to intracellular distribution of QDs in cells and the associated nanoscale effects (6). Previously, we showed that CdSe core/ZnS shell QDs induced cyto- and genotoxicity in human normal lymphocytes as well as human lung cancer cells. Specifically, QD phototoxicity was remarkably increased following UV irradiation via reactive oxygen species (ROS) generation, single- and double-stranded DNA damage, and finally, apoptotic or/and necrotic cell death, which implies potential photodynamic therapy (PDT) applications for lung cancer cells (2). Numerous studies have strived to reduce QD toxicity and render them water-soluble for biological applications; potential methods of reducing toxicity include synthesis of QD-gelatin nanocomposites, cadmium-free QDs, and biofunctionalized (polymer or peptide conjugates) QDs (5,7,8).

Correspondence to: Hai Won Chung, School of Public Health and Institute of Health and Environment, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Korea  
Email: [chunghw@snu.ac.kr](mailto:chunghw@snu.ac.kr)

<sup>†</sup>The two authors contributed equally to this work.

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Curcumin, a natural yellow pigment isolated from turmeric (*Curcuma longa*), is an anti-inflammatory, antioxidant, antimicrobial, and anticancer agent, and has long been used as a food additive and medicinal agent (9-11). Curcumin exerts dual actions, both as an antioxidant and cytotoxicant (12). In addition to its cytotoxic anticancer effects, curcumin reduces oxygen free radicals, prevents lipid peroxidation, and attenuates DNA damage (10). 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- $\kappa$ B) activation, modulating autophagy, and inhibiting tumor angiogenesis (13). 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) (14). 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 (13-15). 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 QDs and curcumin under UVA irradiation on apoptotic cell death in HL-60 cells and the protective effect of curcumin against CdSe/ZnS QD-induced cyto/genotoxicity in human normal lymphocytes were examined.

## MATERIALS AND METHODS

**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 U/ml of each) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. 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  $\times$ 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 U/ml each of penicillin and streptomycin. Lympho-

cytes were stimulated with 1% phytohemagglutinin (PHA; Gibco, Invitrogen, CA, USA) and cultured in a humidified atmosphere at 37°C and 5% CO<sub>2</sub>.

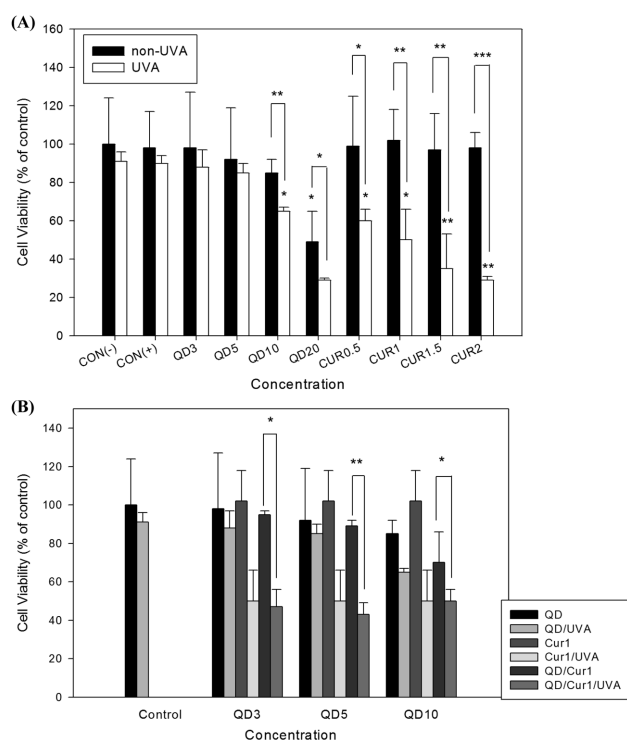
### **Preparation and characterization of CdSe/ZnS QDs.**

CdSe/ZnS QDs dispersed in toluene were supplied by Sigma-Aldrich (St. Louis, MO, USA). In this study, we used the same CdSe/ZnS QD nanoparticles as in our previous study (2). Briefly, we removed the toluene using rotary evaporation (Rotavapor R-210; Buchi Laboratory Equipment, Zurich, Switzerland) and suspended the QDs in distilled water, which was sonicated for 30 min at 4°C 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 nm. Transmission electron microscopy (TEM; 100CX; JEOL, Tokyo, Japan) was used to determine the size and shape of the CdSe/ZnS QDs. Absorption peaks of the CdSe/ZnS QDs were measured using an ultraviolet-visible (UV-Vis) spectrophotometer (V-650; Jasco, Tokyo, Japan), and photoluminescence spectra were detected with a fluorescence spectrophotometer (F-4500; Hitachi, Tokyo, Japan). The QD surface charge was measured in distilled water using a zeta potential analyzer (Zeta plus; Brookhaven Instruments Corp., Holtsville, NY, USA).

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

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

**Single-cell gel electrophoresis assay.** Single-cell gel electrophoresis was performed as described by Singh *et al.* (16). HL-60 cells and human normal lymphocytes were



**Fig. 1.** HL-60 cell viability following treatment with QDs and/or curcumin in the presence or absence of UVA irradiation. Cells were incubated in a 96-well microplate for 24 h, treated with 3, 5, 10, or 20  $\mu\text{g/ml}$  QDs or 0.5, 1, 1.5, or 2  $\mu\text{g/ml}$  curcumin, respectively (A) and co-exposed to 3, 5, or 10  $\mu\text{g/ml}$  QDs and 1  $\mu\text{g/ml}$  curcumin (B). Untreated control, CON(-); negative control (treated with DMSO), CON(+). Cell viability was determined by the WST 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. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

treated with curcumin and/or CdSe/ZnS QDs for 1 hr and 3 hr, respectively, followed by UVA ( $2 \text{ J/cm}^2$ ) exposure. After two PBS washes, the cells were maintained at  $4^\circ\text{C}$  to prevent DNA repair. Slides were prepared according to the method of Singh (16). Briefly, images of 60 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 nm excitation filter and 590 nm barrier filter.

**Cytokinesis-block micronucleus assay.** The cytokinesis-block micronucleus (MN) assay was performed as described by Fenech (17). HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% FBS, incubated with curcumin and/or CdSe/ZnS QDs for 1 hr and 3 hr, respectively, and then irradiated with UVA. Cytochala-

sin B ( $4 \mu\text{g/ml}$ ; Sigma-Aldrich, St. Louis, MO, USA) was added 20 hr after the start of culture, and the incubation was continued for an additional 28 hr.

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

After a total incubation period of 48 hr for HL-60 cells and 72 hr for whole blood lymphocytes, the cells were harvested, treated with hypotonic 0.075 M KCl solution for 1 min, and washed twice with fixative solution (methanol : acetic acid, 3 : 1). Air-dried cell preparations were stained with Giemsa solution (5%). In total, 1,000 binucleated cells with well-preserved cytoplasm were scored according to standard criteria (18). In addition, cytokinesis-block proliferation index (CBPI) 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.

**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 hr and QDs for 3 hr, and then irradiated with UVA. Thereafter the medium was discarded through centrifugation, and fresh culture medium containing 20  $\mu\text{M}$  DCFDA was added in the dark. The cells were incubated for 20 min at  $37^\circ\text{C}$  and fluorescence distribution was monitored by flow cytometry (FACS Caliber, Becton-Dickinson, CA, USA).

**Measurement of apoptosis.** Apoptosis induction by curcumin and/or CdSe/ZnS QDs 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 QDs were collected, washed twice with PBS, and fixed with 70% cold aqueous ethanol for 24 hr and then stored at  $-20^\circ\text{C}$  for at least 24 hr. Cells were washed twice with PBS and cell pellets were stained with PI solution containing RNase A (10 mg/ml) and PI (10 mg/ml) in PBS. The cell suspension was incubated in the dark at room temperature for 30 min, and DNA content was assayed by flow cytometry (FACS Caliber, Becton-Dickinson, CA, USA).

**Statistical analysis.** The effects of curcumin and/or CdSe/ZnS QDs 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

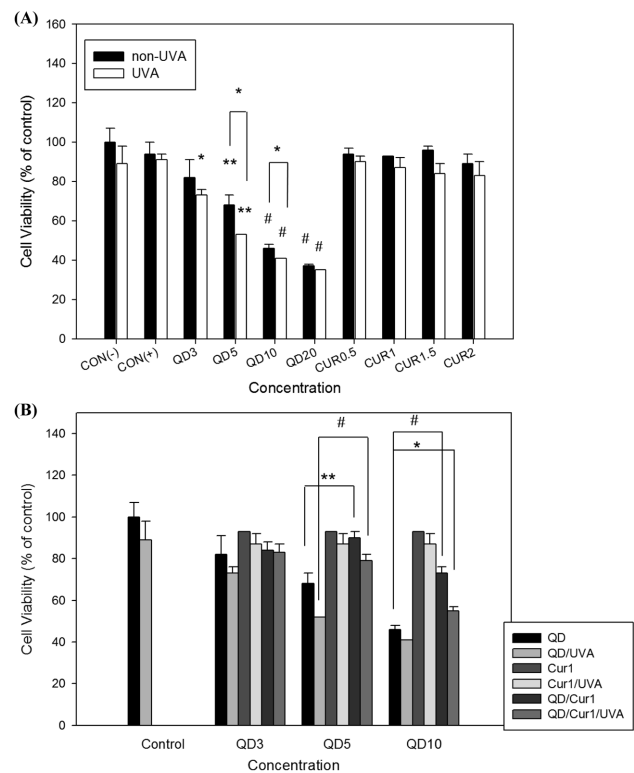
as means  $\pm$  standard deviation (S.D.).

## RESULTS

**Physicochemical characterization of QDs.** As we reported previously, the average size of core-shell type CdSe/ZnS QDs as determined by DLS (particle size, 3.3 nm;  $\lambda_{em}$ , 530 nm) was  $105 \pm 15.55$  nm, indicating that QD particles aggregate in an aqueous environment. The CdSe/ZnS QD UV-Vis absorption spectrum showed broad continuous absorption at wavelengths ranging from UV to visible. The zeta potential measurement in distilled water showed a  $\zeta$  potential of  $+40.58 \pm 7.97$  mV, confirming the colloidal stability of CdSe/ZnS QDs (2).

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

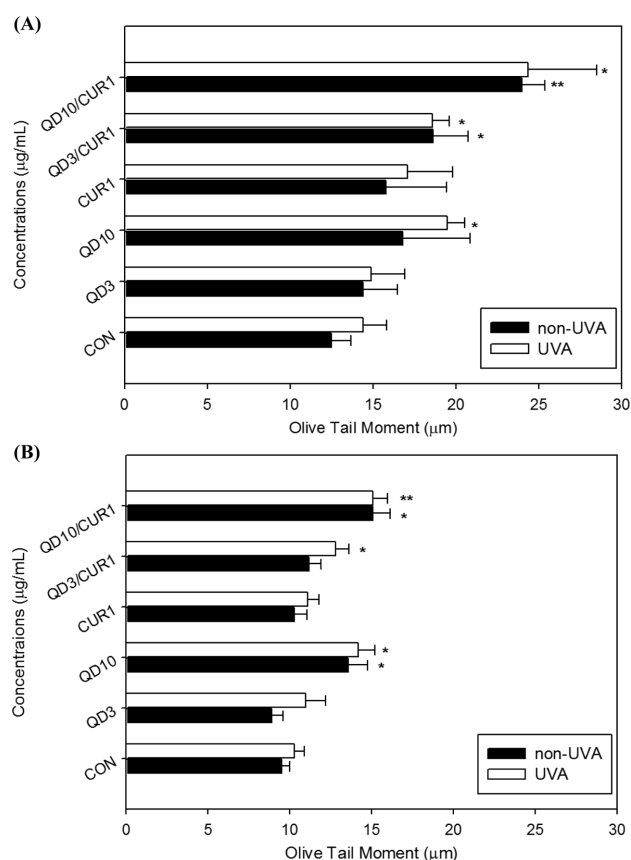
**Effect of curcumin on normal lymphocyte viability following QD treatment under UVA irradiation.** To assess the effect of curcumin on QD-induced cytotoxicity in normal lymphocytes isolated from whole blood, the WST assay was used. CdSe/ZnS QD treatment decreased the viability of normal lymphocytes in a concentration-dependent manner, but curcumin with/without UVA irradiation had a similar effect on normal lymphocytes as in control groups (Fig. 2A). Curcumin treatment protected against CdSe/ZnS QD-induced cytotoxicity in normal lymphocytes, as seen in Fig. 2B. CdSe/ZnS QDs (5 and 10  $\mu$ g/ml) in combination with 1  $\mu$ g/ml curcumin increased cell viabilities by 90% and 73% respectively, which were reduced by UVA irradiation to 79% and 55%, as compared with CdSe/ZnS QDs alone (68% and 46%, respectively). Although UVA irradiation decreased cell viability somewhat, the protective effect of curcumin on normal lymphocytes was statistically significant ( $p < 0.05$ ).



**Fig. 2.** Cultured human normal lymphocyte cell viability following treatment with QDs and/or curcumin in the presence or absence of UVA irradiation. Cells were incubated in a 96-well microplate for 24 h, treated with 3, 5, 10, 20  $\mu$ g/ml QDs or 0.5, 1, 1.5, 2  $\mu$ g/ml curcumin, respectively (A) and co-exposed to 3, 5, 10  $\mu$ g/ml QDs and 1  $\mu$ g/ml curcumin (B). CON(-), untreated control; CON(+), negative control (treated with DMSO). Cell viability was determined by the WST 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. \*  $p < 0.05$ , \*\*  $p < 0.01$ , #  $p < 0.001$ .

icant ( $p < 0.05$ ).

**Effect of curcumin on CdSe/ZnS QD-induced DNA damage as indicated by the comet assay.** We evaluated genotoxicity induced by curcumin and/or CdSe/ZnS QDs with/without UVA irradiation in HL-60 cells and normal human lymphocytes using the comet assay, in which the extent of DNA damage is represented by OTM (Fig. 3). Significant DNA damage was evident in HL-60 cells treated with 10  $\mu$ g/ml QDs plus UVA irradiation (OTM:  $19.45 \pm 1.05$ ), cells treated with 3  $\mu$ g/ml QDs and curcumin (OTM:  $18.62 \pm 2.11$ ), and cells treated with 3  $\mu$ g/ml QDs and curcumin plus UVA (OTM:  $18.59 \pm 1.02$ ) as compared with the control group. Combination treatment with curcumin and QDs induced statistically significant single-stranded DNA damage in HL-60 leukemia cells (Fig. 3A).



**Fig. 3.** Single-stranded DNA breaks in HL-60 cells treated with QDs 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  $\pm$  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.

OTM in normal lymphocytes treated with 3  $\mu\text{g/mL}$  CdSe/ZnS QDs and 1  $\mu\text{g/mL}$  curcumin was slightly increased as compared with the control group (OTM:  $11.2 \pm 0.72$  for 3  $\mu\text{g/mL}$  QDs plus curcumin and  $9.6 \pm 0.51$  for control). In this study, no protective effect of curcumin against QD-induced DNA single strand breaks was found in normal lymphocytes (Fig. 3B).

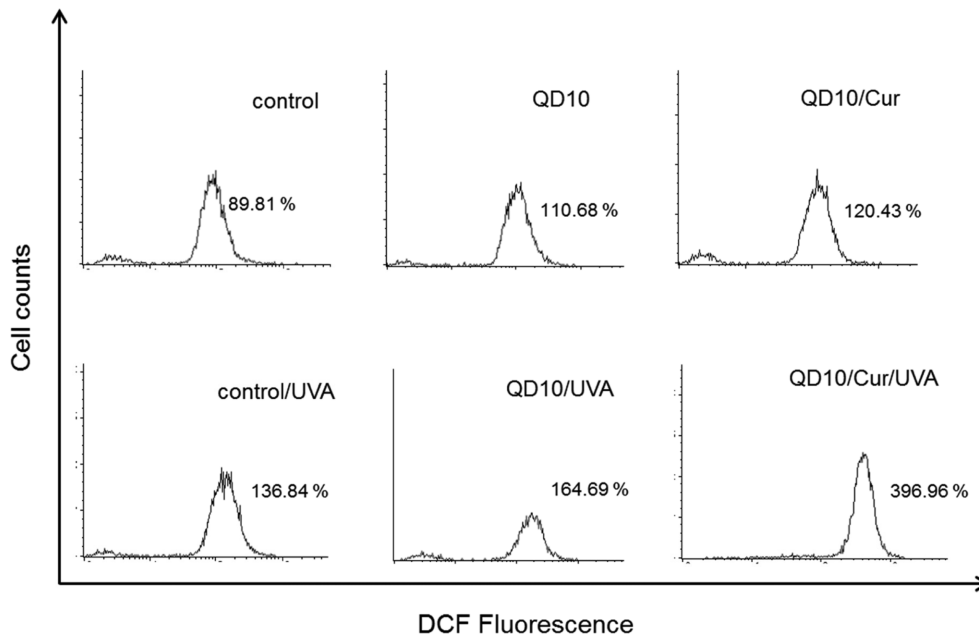
**Effect of curcumin on CdSe/ZnS QD-induced DNA damage by micronucleus assay.**

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

**Table 1.** Induction of micronuclei (MN) in HL-60 cells and human normal lymphocytes treated with QDs and/or curcumin with/without UVA irradiation

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

Micronuclei induction was measured by cytokinesis block micronucleus assay. MN, micronuclei; BNCs, binucleated cells; CBPI, cytokinesis-block proliferation index. Values represent means  $\pm$  standard deviation (S.D.). Data were analyzed statistically using Kruskal-Wallis tests and Mann-Whitney *U*-tests. \*  $p < 0.05$  compared to the control.



**Fig. 4.** Effect of curcumin on reactive oxygen species (ROS) generation in QD-treated HL-60 cells under UVA irradiation. HL-60 cells were pretreated with curcumin for 1 h, prior to treatment with QDs. After 3 h, the cells were irradiated with UVA light ( $2 \text{ J/cm}^2$ ) and ROS generation was assayed by flow cytometry. Significant differences were observed following treatment with  $10 \mu\text{g/ml}$  QDs and  $1 \mu\text{g/ml}$  curcumin plus UVA irradiation as compared with the other treatment groups.

**Effect of curcumin on CdSe/ZnS QD-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 QD treatment alone, QD treatment with UVA exposure, and QD plus curcumin co-treatment; ROS formation significantly increased in cells irradiated with UVA in combination with QD and curcumin co-treatment. ROS generation in cells treated with  $10 \mu\text{g/ml}$  QDs and curcumin plus UVA irradiation was increased fourfold compared to the control group (Fig. 4). These results indicate that UVA irradiation enhances ROS generation in HL-60 cells treated with a combination of CdSe/ZnS QDs and curcumin.

**Assessment of apoptosis.** We next conducted apoptosis assays to investigate the mode of CdSe/ZnS QD and/or curcumin-induced cell death in the presence or absence of UVA irradiation. HL-60 cells treated with QDs alone and/or exposed to UVA, co-treatment of QDs with curcumin and/or UVA exposure, and co-treatment of QDs with curcumin and/or UVA exposure for 24 hr resulted in a concentration-dependent increase in apoptotic cell death, which increased to  $\sim 32.73\%$  following QD and curcumin treatment plus UVA irradiation, as compared to  $6.98\%$  for the untreated control. Apoptotic cell death occurred in  $\sim 16.97\%$  of normal human lymphocytes following treatment with the same concentrations as in the HL-60 cells, as compared to  $11.88\%$

**Table 2.** The percentages of apoptotic cells measured using flow cytometry in HL-60 cells and normal lymphocytes

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

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

in the untreated controls (Table 2). These results clearly indicate that the QD and curcumin combined treatment enhances apoptosis in cancer cells, whereas the combination has a protective effect in normal human lymphocytes.

## DISCUSSION

Semiconductor nanocrystal QDs, which display unique

optical and electrical properties, are being used in many biological applications. Although QDs 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 (19). In a previous study, we established that CdSe/ZnS QDs induced cyto-/genotoxicity through DNA damage, ROS induction, and activation of the apoptotic signaling pathway in A549 cells; we also proposed that they were a potential photosensitizer in photodynamic therapy (2). The limitation of QD use due to their toxicity requires more effective cancer treatments that are selectively targeted toward cancer cells without damaging healthy tissue. Here, we used a combined QD 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 antimutagenic agent that protects normal tissues (10). 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 QDs 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, we observed distinct sensitivities to the QD and curcumin combined treatment, indicating the selective role of curcumin. Cell death and apoptosis were enhanced by curcumin combined with QDs plus UVA exposure in HL-60 cells (Fig. 1 and Table 2). Unlike HL-60 cells, curcumin had a protective effect against QD-induced toxicity on normal lymphocytes (Fig. 2 and Table 2). 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 QDs and curcumin with UVA exposure (Fig 4), while curcumin attenuated the QD-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 (20-22). ROS caused by QD and curcumin exposure under UVA irradiation clearly increased single- and double-stranded DNA breaks as compared to QD treatment alone, QD/UVA co-exposure, curcumin treatment alone, curcumin/UVA co-exposure, or QD and curcumin co-treatment in HL-60 cells (Fig. 3A and Table 1). Although the protective effect of curcumin was not statistically significant, the micronuclei frequency was lower in QD and curcumin-co-treated than in QD-treated cultured normal lymphocytes. Our results suggest that curcumin did not produce cyto-/genotoxicity, but protected normal lymphocytes from QD-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 QDs in HL-60 cancer cells. Furthermore, the reduced cell viability was precipitated by UVA irradiation

with QDs 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 phase 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 (23-26).

Taken together, our data suggest that QD 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 QDs and curcumin.

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