Transient Metals Enhance Cytotoxicity of Curcumin: Potential Involvement of the NF-κB and mTOR Signaling Pathways

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Abstract. *Background/Aim: Curcumin has been recognized as a metal-binding compound and an anticancer agent, yet the involvement of metals in the anticancer action of curcumin remains unclear. The present study examined the role of transient metals in curcumin-induced cytotoxicity in cancer cells. Materials and Methods: Metal-binding activity and cytotoxicity of curcumin were examined in human cancer lines with cell viability assay, confocal microcopy, Western blot, and measurement of hydrogen peroxide generation. Results: It was found that Cu (II) most significantly potentiated the cytotoxicity of curcumin among the metals tested. The combination of curcumin and Cu (II) did not generate reactive oxygen species and vitamin E did not block the cytotoxicity. Curcumin plus Cu (II) enhanced intracellular copper levels and potentiated curcumin-induced suppression of the nuclear factor kappa B (NF-κB) pathway, as well as alterations of mammalian target of rapamycin-raptor (mTOR) signaling. Conclusion: Transient metals enhance the cytotoxicity of curcumin, likely through targeting of the NF-κB and mTOR signaling pathways.*

A number of metal-binding compounds have been considered as potential anticancer agents, and some of them are currently being tested in clinical trials (1-3). These metal-binding compounds exert their cytotoxicity on cancer cells through several cellular mechanisms (4). Traditionally, metal binding compounds act as chelators that remove cellular metals which are essential for cell survival, thereby leading to apoptotic cell death (5, 6). Another group of metal binding compounds that are toxic to cancer cells by acting as metal ionophores has recently been identified (4). It is apparent that metal-binding compounds can also act as "shuttles" that form complexes with metals which are cytotoxic to cancer cells (4).

Curcumin is an active ingredient found in the spice turmeric (*Curcuma longa Linn.*). Its anticancer properties have been recognized in experimental model systems, and it is currently being tested in a number of clinical trials (7). While curcumin has been shown to act as an antioxidant (8), an inhibitor of nuclear factor kappa B (NF-κB) (9, 10), and a modulator of the mammalian target of rapamycin-raptor (mTOR) signaling pathway (11), the mechanisms of its anticancer action remain to be further elucidated. Importantly, curcumin has been demonstrated to be an iron chelator (12) and can form a complex with copper in test tubes (13). These studies clearly indicate that curcumin is a metal-binding compound. However, the involvement of the metal-binding activity of curcumin in its anticancer action has been less investigated. As metals have been shown to be involved in carcinogenesis, and are essential for the cells to survive, their contributions to the anticancer action of curcumin is an interesting matter for investigation.

This study reports that transient metals, especially Cu (II), enhance the cytotoxicity of curcumin, likely through NF-κB and mTOR-mediated signaling pathways. Based on the criteria used to categorize metal-binding compounds, the results suggest that curcumin acts as a metal ionophore (4). These findings offer a new insight into the behavior and mechanism of the anticancer action of curcumin.

Materials and Methods

Materials. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) reagent was obtained from Promega (Madison, WI, USA); 4EBP1 and p-4EBP1 antibodies from Cell Signaling Tech. (Danvers, MA, USA); p70S6 kinase and p-p70S6 kinase antibodies from Santa Cruz Biotech., Inc. (Santa Cruz, CA, USA); GAPDH antibody from ProMab Biotech (Richmond, CA, USA). Curcumin (Diferuloylmethane), $CuCl_2$, $FeCl_3$, $ZnCl_2$, and all other chemicals and reagents were analytic grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

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Cell lines and cell viability assay. The ovarian cancer line, A2780, was provided by Dr. Stephen Howell (University of California, San Diego, CA, USA). The human breast cancer cell line, MCF-7 was purchased from American Type Tissue Culture (ATCC, Manassas, VA, USA). Cells were cultivated in RPMI-1640 (MCF-7, A2780) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were grown in a humidified environment of 5% CO_2 at 37˚C, and propagated twice a week. For the cell viability assay, cells were seeded at 3,000-5,000 cells per well in a 96-well plate and exposed to various drugs or combinations of drugs for 72 hours. The cell viability was then assessed using an MTS reagent as described previously (14). Briefly, 20 μl of MTS reagent was added to each well and incubated at 37˚C for one hour before the absorbance of each well was recorded at 490 nm. Data are expressed as a percentage of the values obtained from the untreated control cells.

Western blot analysis. Western blot was performed as previously described (15). Briefly, A2780 cells were seeded at $1.8x10^6$ cells/dish, grown in 100-mm dishes and treated with curcumin and copper at indicated concentrations and times. Cells were then lysed in a buffer containing 50 mM Tris HCl (pH 7.8), 100 mM NaCl, 5 mM Na-EDTA, 0.1% SDS, 1 mM PMSF, 1% Triton-X-100, and 2.5% glycerol. The cell lysates were sonicated for 1 min and incubated at 4˚C for one hour before being subjected to centrifugation at 13.000 rpm for 15 minutes. The supernatants were collected and the protein concentration was determined. Protein (40 μg) from each sample was loaded into each well of a 10% SDS-PAGE gel and transferred onto a polyvinylidene fluoride membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK). The membrane was blocked with 5% milk diluted in a mixture of Tris buffered saline (TBS) and 0.1% Tween-20, pH adjusted to 7.4 (TBST) overnight at 4˚C and incubated with the appropriate primary and secondary antibodies the following morning. Chemiluminescence (GE Healthcare) was used to detect the signal.

Hydrogen peroxide assay. Hydrogen peroxide concentrations were measured using a colorimetric assay (BioVision, Mountain View, CA, USA). Briefly, $CuCl_2$ and curcumin were mixed in PBS (phosphate buffered saline) at indicated concentrations (pH 7.4) at room temperature. The reaction mixtures (30 μl) were immediately added into each well of a 96-well plate; 20 μl of assay buffer were added to each well. Standards were generated by adding H_2O_2 in place of the reaction mixture at concentrations of 0, 1, 2, 3, 4, 5 nmol/well. Fifty μl of a reaction mixture, containing OxiRed™ Probe and horseradish peroxidase, were then added and incubated for 10 min at room temperature. The optical density of the solution was measured at 570 nm.

Intracellular copper detection. Intracellular copper levels were monitored with a fluorescent tracer, Phen Green FL probe, following the manufacturer's protocol (excitation 490/20 nm and emission 528/38 nm, Invitrogen, Carlsbad, CA, USA). Following entrance into the cells, Phen Green FL fluoresces if it is not bound by copper. Copper binding quenches the fluorescence of the probe. Cells were treated with $CuCl_2$ alone or in combination with curcumin for 1 hour at indicated concentrations. The medium was then replaced with fresh medium containing 5 μM Phen Green FL. After 30 min incubation, the medium was removed and the cells were thoroughly washed with HBSS (Hanks balanced salt solution) and viewed under the Leica TCS NT Confocal Microscope using 63× Plan APO 1.2 NA.

Table I. *Transient metals enhance cytotoxicity of curcumin in A2780 cells. Cells were treated with increasing concentrations of curcumin in the presence or absence of 30 μM of $FeCl_3$, $ZnCl_2$, or $CuCl_2$. Cell viability was analyzed with the MTS assay and data (mean±SE, n=3) are expressed as percentages of the MTS level detected in untreated control cells.*

Curcumin (μM)	No metals	+ $FeCl_3$	+ $ZnCl_2$	+$CuCl_2$
0	100	81±4	86±6	74±1
1	91±3	79±7	89±4	76±2
3	89±4	71±6	87±6	62±7
5	83±6	62±12	64±13	56±5
10	68±1	51±7	61±8	28±3
30	41±6	14±1	32±3	6±3
100	16±4	-	-	-

Statistical analysis. The differences among experimental groups were determined using One-way ANOVA analysis (GraphPad Prism version 4.00 for Windows; GraphPad Software, San Diego CA, USA).

Results

Curcumin alone inhibited cell viability of A2780 cells in a concentration-dependent manner (Figure 1A). Cu (II) alone also inhibited cell viability, while treatments with Zn (II) or Fe (III) did not cause any significant change (Figure 1B-D). These same metals were combined with curcumin and the effects on cell viability were examined. Combination of curcumin with Cu (II) showed a clear enhancement of cytotoxicity, whereas a moderate increase in cytotoxicity was observed when combined with Zn (II) or Fe (III) as compared to curcumin alone (Table I). The data therefore show that curcumin combined with copper is more effective at killing cancer cells. The same combination treatments also led to enhanced cytotoxicity in a human breast cancer line MCF-7 (Figure 2).

Since Cu (II) is redox active and a previous study has suggested that Cu (II) interacts with synthetic compounds to generate hydrogen peroxide (16), the combination of copper and curcumin was tested to investigate if the cytotoxic mechanism proceeds in a similar manner. Using the *N*-acetylcysteine and Cu (II) combination as a positive control, it was found that copper and curcumin do not generate hydrogen peroxide, suggesting that the mechanism of cell death induced by the curcumin-copper combination occurs through a different mode of action (Figure 3A). Furthermore, pre-treatment of the cells with vitamin E did not attenuate or block the cytotoxicity induced by curcumin and Cu (II), further indicating that this effect is not mediated through oxidative stress (Figure 3B). Vitamin E blocked docosahexaenoic acid-induced cytotoxicity (data not shown), results similar to a previous report (17).

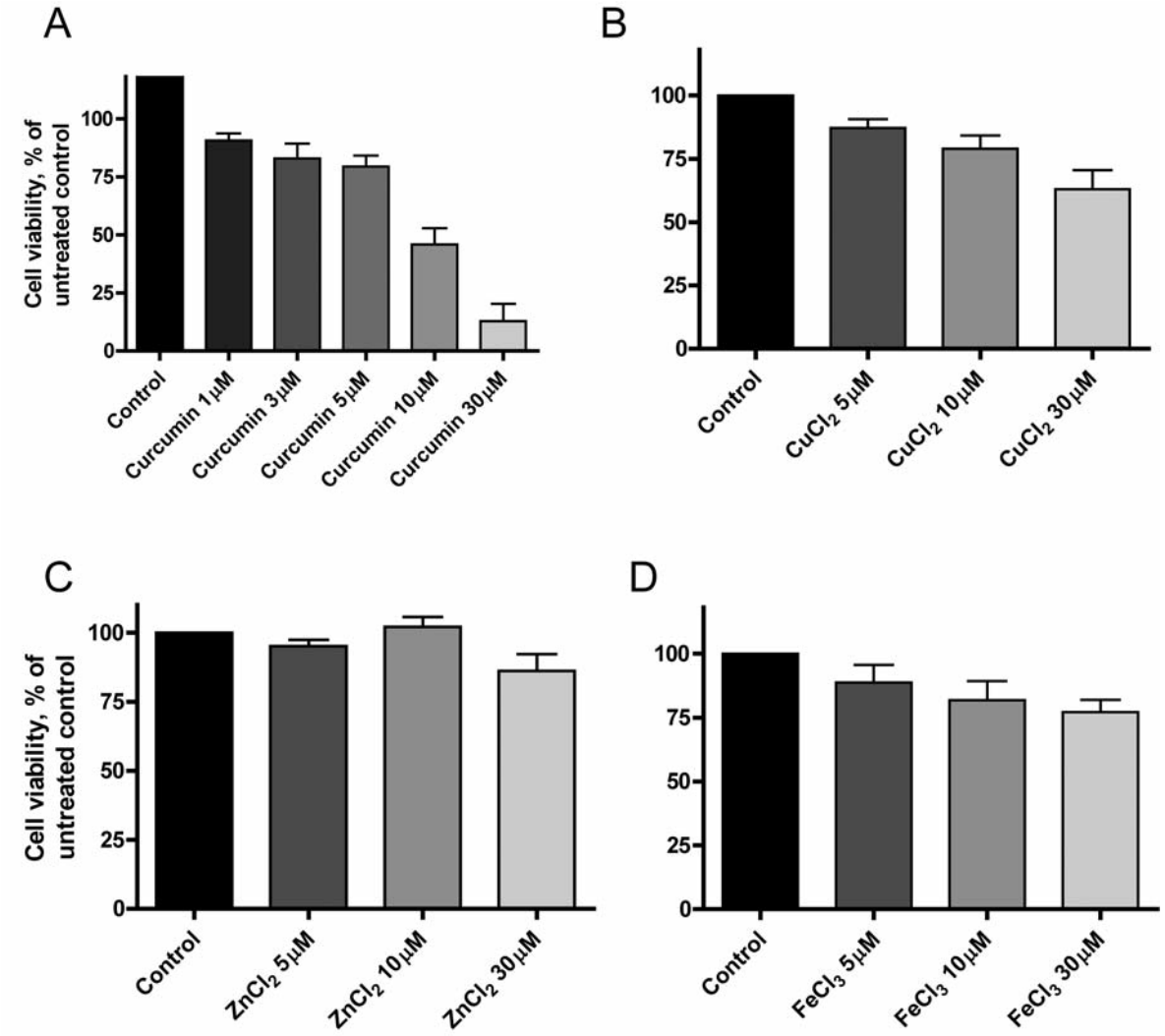

Figure 1. *Effects of curcumin and transient metals on the viability of A2780 cells. Cells were treated with various concentrations of curcumin (A), $CuCl_2$ (B), $ZnCl_2$ (C), or $FeCl_3$ (D) for 72 hours. Cell viability was determined with the MTS assay. Data (mean±SE, n=3) are presented as percentages of the MTS level detected in untreated control cells.*

To understand why Cu (II) enhances the cytotoxicity of curcumin, the copper intake in the cells was tracked using a fluorescent tracer, the Phen Green FL probe, a copper ion indicator tagged with green fluorescence. Cu (II) intake was observed in live cells under confocal microscopy. Treatment with curcumin alone showed no significant effect on the intracellular free copper level, and $CuCl_2$ alone showed a slight reduction of the fluorescence (Figure 4); however, the combination of these two compounds showed a significant quenching in the fluorescence intensity, indicating that curcumin brings copper into the cells and thus acts as a copper ionophore.

Previous studies showed that curcumin targets the NF-κB pathway to attenuate cell viability (18). It has also recently been reported that metal ionophores attenuate NF-κB activity in cancer cells (15). Based on these previous observations, the activity of NF-κB when the cells were treated with the Cu (II)-curcumin combination was tested. Clioquinol plus zinc, which is known to down-regulate NF-κB signaling (15), served as a control.

The reporter gene assay indicated that the Cu (II)-curcumin combination significantly inhibits the NF-κB signaling pathway compared to either compound used alone (Figure 5A); these results support the involvement of this pathway for the potentiated cytotoxicity. This was confirmed by Western blot analysis of the phosphorylation of IkBα, the well-established NF-κB inhibitory protein. An up-regulation of IkBα phosphorylation in cells treated with the Cu (II)-curcumin combination, but not the control or single-agent

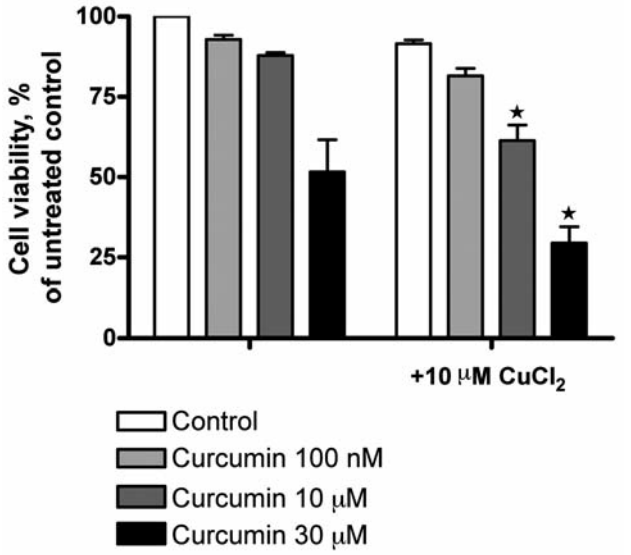

Figure 2. *Effects of the combination of curcumin and copper on the viability of MCF-7 cells. MCF-7 cells were treated with various concentrations of curcumin in the presence or absence of 10 μM $CuCl_2$ for 72 hours. Cell viability was determined with the MTS assay. Data (mean±SE, n=3) are presented as percentages of the MTS level detected in untreated control cells. *p<0.05, compared with curcumin-treated cells in the absence of CuCl2, using one-way ANOVA followed by Dunnett's analysis.*

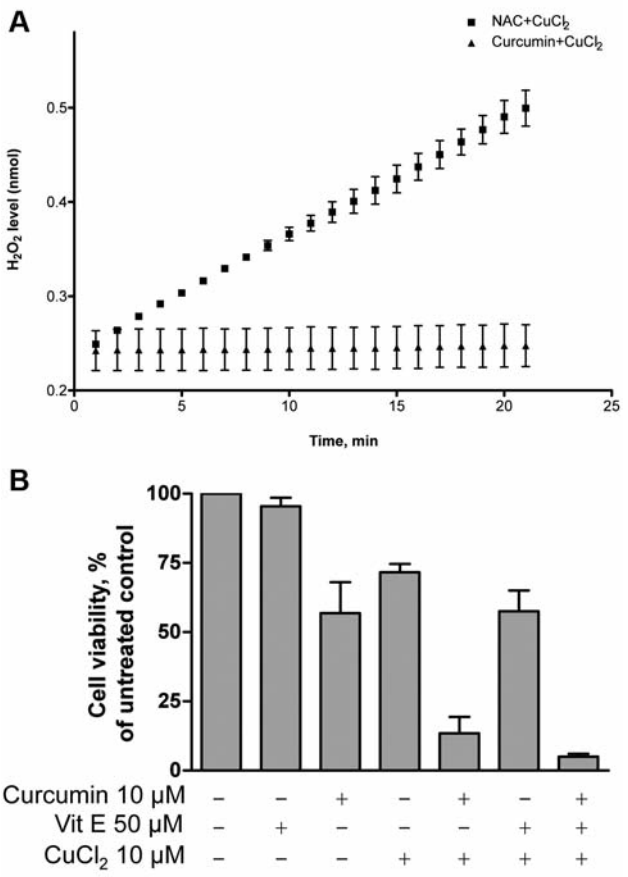

Figure 3. *The cytotoxicity of curcumin plus CuCl2 is not mediated through oxidative stress. A: 10 μM curcumin was mixed with 10 μM CuCl2 and the amount of hydrogen peroxide generated was measured after 30 minutes. 1 mM N-acetylcysteine plus 10 μM $CuCl_2$ was used as a positive control. Data are expressed as nmol of hydrogen peroxide generated in the reaction mixture (means±SD, n=3). B: A2780 cells were pretreated with 50 μM vitamin E for 20 min prior to addition of curcumin plus $CuCl_2$ for 72 hours at indicated concentrations. Cell viability was determined with the MTS assay. Data (mean±SE, n=2) are presented as percentages of the MTS level detected in untreated control cells.*

treatments, further indicates the inhibition of NF-κB signaling in this model system (Figure 5B).

Another recently identified cellular target of curcumin is the mTOR signaling pathway (11). In the current study, it was found that the combination of curcumin and Cu (II) enhances expression of both total and phospho-p70-S6 kinase in A2780 cells, indicating disruptions of the mTOR signaling pathway (Figure 6). Curcumin alone down-regulates expression of phospho-4EBP1 (data not shown), another component of mTOR signaling; an observation consistent with a previous report (11).

The results shown in Figures 5 and 6 thus suggest that copper plays an important role in the action of curcumin on both NF-κB and mTOR signaling pathways in cancer cells, which most likely explains the enhanced cytotoxicity by the combination.

Discussion

While curcumin has been well-recognized as a metal-binding compound and extensively studied in experimental cancer therapeutic model systems, the involvement of metals in the anticancer of curcumin action has been less investigated. The present study demonstrated that Cu (II) significantly potentiates the cytotoxicity of curcumin in cancer cells, likely through targeting the NF-κB and mTOR signaling pathways. These experimental results provide novel insight into the anticancer activity of curcumin.

Cu (II) is known as a redox reactive agent. The fact that Cu (II) enhances the cytotoxicity of curcumin more effectively when compared to Fe (III) and Zn (II), led us to the prediction that the enhanced cytotoxicity could be a result of augmented oxidative pressure. Indeed, previous studies have shown that Cu (II) could interact with synthetic compounds generating reactive oxygen species (16) (our unpublished data). However, the combination of curcumin and Cu (II) does not lead to hydrogen peroxide generation in test tubes, indicating that copper enhances cytotoxicity of

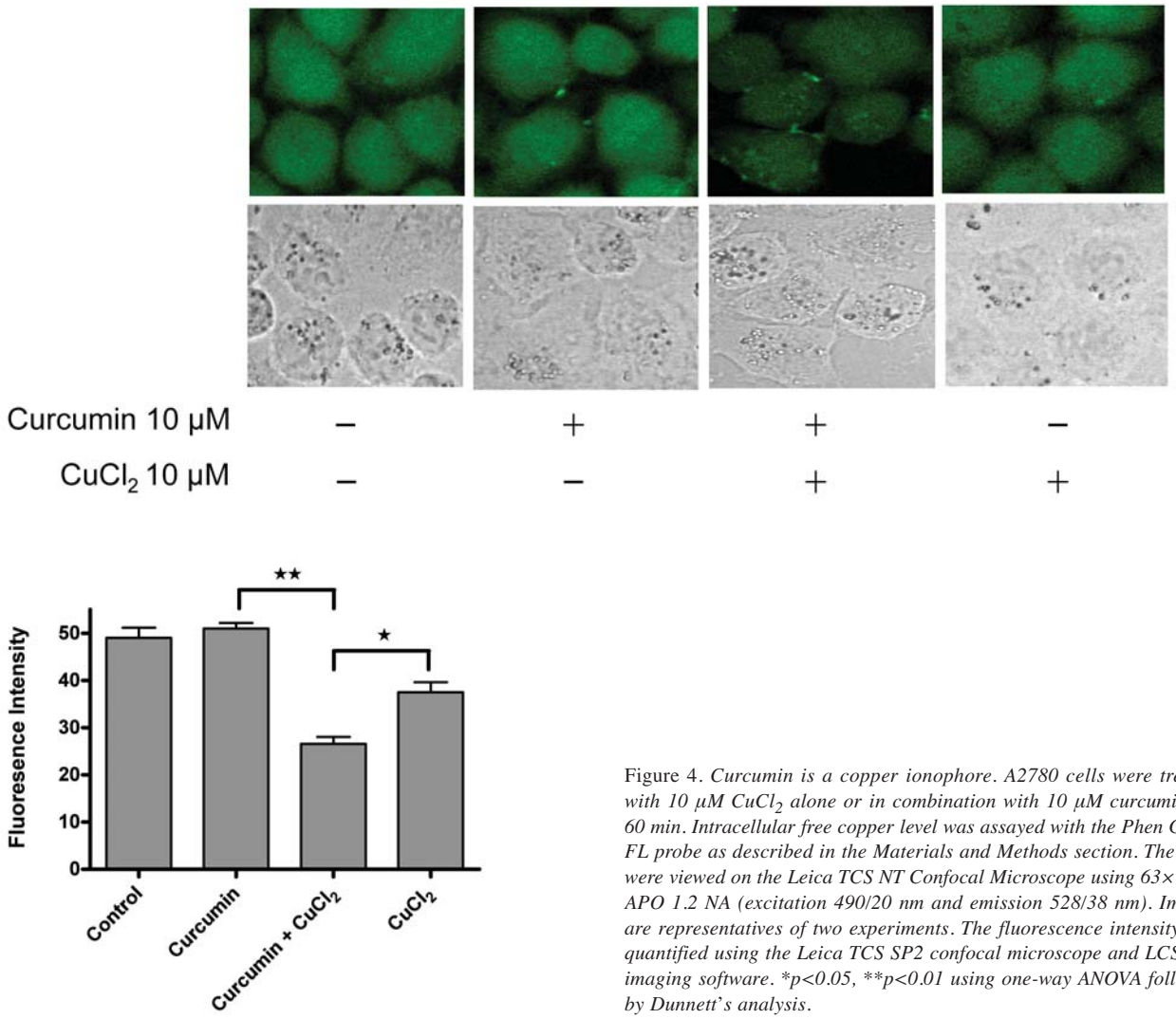

Figure 4. *Curcumin is a copper ionophore. A2780 cells were treated with 10 μM $CuCl_2$ alone or in combination with 10 μM curcumin for 60 min. Intracellular free copper level was assayed with the Phen Green FL probe as described in the Materials and Methods section. The cells were viewed on the Leica TCS NT Confocal Microscope using 63× Plan APO 1.2 NA (excitation 490/20 nm and emission 528/38 nm). Images are representatives of two experiments. The fluorescence intensity was quantified using the Leica TCS SP2 confocal microscope and LCS Lite imaging software. *p<0.05, **p<0.01 using one-way ANOVA followed by Dunnett's analysis.*

curcumin through other mechanisms of action. This is consistent with a previous study suggesting that a synthetic complex of curcumin and Cu (II) acts as an antioxidant (19). The failure of vitamin E in blocking the cytotoxicity of curcumin and Cu (II) further supports the idea that oxidative stress is not involved in this event.

The metal-binding property of curcumin has been previously recognized. It is reported that curcumin acts as an iron chelator that has higher affinity for Fe (II) versus Fe (III) (12). Barik *et al*. described the formation of a curcumin and Cu (II) complex that possesses superoxide dismutase activity (19). A recent study reported that curcumin binds to copper (II) with a Kd in the low micromolar range (13). The current experiment with the intracellular free copper tracer indicates that curcumin brings copper into the cells and releases it thereafter, resulting in an elevated intracellular free copper level. The dissociation of copper ions from curcumin in the cells supports the previous finding that the binding affinity of curcumin for copper is relatively moderate (13). This assertion is supported by the observation that addition of copper enhances the cytotoxicity of curcumin rather than attenuating it, a phenomenon fitting into a recently established biological definition of metal ionophores (4).

The NF-κB and mTOR signaling pathways are established cellular targets for cancer therapy (20, 21). Curcumin has been previously found to target both pathways (10, 11), which are believed to be associated with the anticancer activity of curcumin (18). In order to understand further how Cu (II) enhances the cytotoxicity of curcumin, whether Cu (II) acts in concert with curcumin to regulate the NF-κB and mTOR signaling pathways was investigated. Addition of Cu (II) potentiated the action of

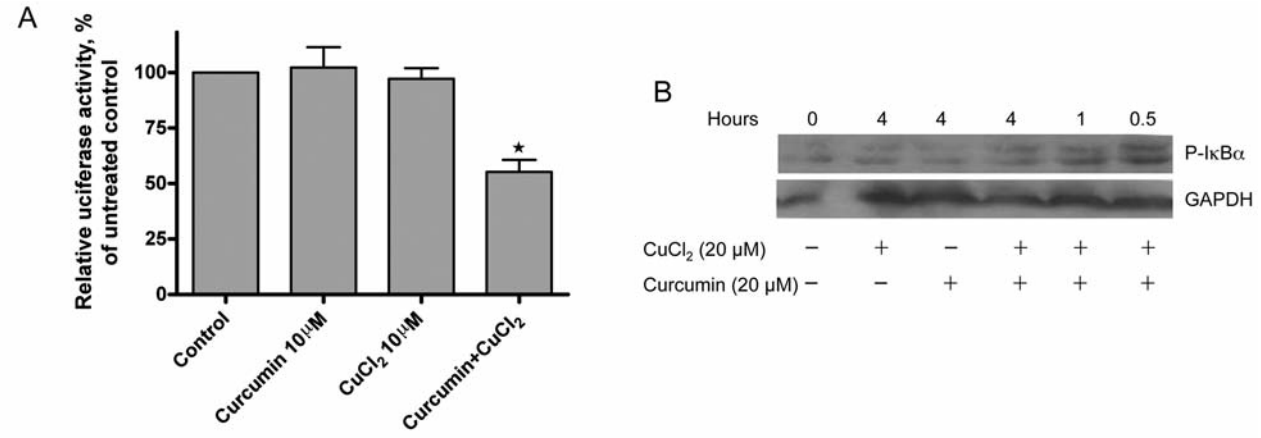

Figure 5. *Effects of curcumin plus $CuCl_2$ on NF-κB activity in A2780 cells. A: Cells were co-transfected with NF-κB Luc and PRL-TK Renilla reporter constructs and treated with 10 μM curcumin alone or in combination with 10 μM $CuCl_2$ for 1 hour. Cell lysates were analyzed using the dual luciferase assay kit. The data (mean±SE, n=2-3) are presented as relative luciferase levels. *p<0.05, compared with control cells using one-way ANOVA followed by Dunnett's analysis. B: Total protein was extracted from A2780 cells that had been treated with 20 μM curcumin alone or in combination with 20 μM $CuCl_2$ for the indicated times. The proteins were separated on an SDS PAGE gel, transferred, and immunoblotted against specific antibodies for IkBα and GAPDH. Representative images of two experiments are shown.*

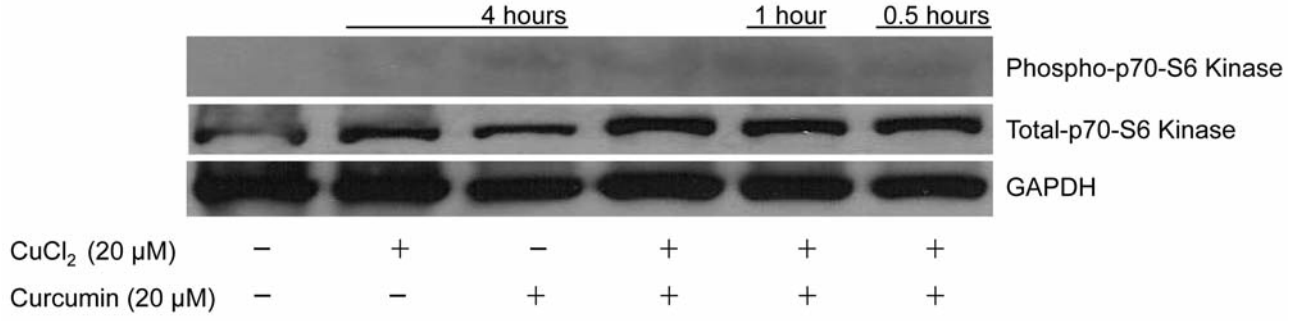

Figure 6. *Effects of curcumin plus $CuCl_2$ on mTOR signaling. Total protein was extracted from A2780 cells that had been treated with 20 μM curcumin alone or in combination with 20 μM $CuCl_2$ for the indicated times. The proteins were separated on an SDS PAGE gel, transferred, and immunoblotted against specific antibodies for p-p70-S6, p70-S6, and GAPDH. Representative images of two experiments are shown.*

curcumin on both pathways, strongly suggesting that Cu (II) potentiates this anticancer action through the targeting of NF-κB and mTOR signaling. It has been previously demonstrated that an increase in intracellular copper level leads to an inhibition of proteasomes (22), which in turn could result in the accumulation of IκBα proteins and inhibition of NF-κB signaling (21). This may explain why the combination of copper and curcumin down-regulates NF-κB signaling more than either one used alone, because curcumin acts as a Cu (II) ionophore, elevating the intracellular Cu (II) level. However, the involvement of copper in modifying mTOR signaling has not been previously described. In this study it was found that Cu (II) enhances the action of curcumin on mTOR signaling, thus providing novel information on the regulation of mTOR signaling by copper in human cancer cells. It remains to be investigated whether the enhancement of curcumin-induced alterations of mTOR signaling by copper is regulated through proteasome inhibition.

It is well documented that copper levels in the plasma of cancer patients and in tumor tissues is significantly higher than those in healthy individuals and normal tissues (23). This provides an opportunity to selectively target tumor cells by copper chelation (5) or copper ionophoring (4, 22). The findings from the present study indicate that curcumin is one of the compounds that may be used to achieve this goal. Because curcumin is currently undergoing clinical trials for the treatment of various tumors, this new understanding of the anticancer activity of curcumin could facilitate further development of this compound as an anticancer agent.

Acknowledgements

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References

1 Huang R, Wallqvist A and Covell DG: Anticancer metal compounds in NCI's tumor-screening database: putative mode of action. Biochem Pharmacol *69*: 1009-1039, 2005.
2 Redman BG, Esper P, Pan Q, Dunn RL, Hussain HK, Chenevert T, Brewer GJ and Merajver SD: Phase II trial of tetrathiomolybdate in patients with advanced kidney cancer. Clin Cancer Res *9*: 1666-1672, 2003.
3 Richardson DR: Therapeutic potential of iron chelators in cancer therapy. Adv Exp Med Biol *509*: 231-249, 2002.
4 Ding WQ and Lind SE: Metal ionophores - An emerging class of anticancer drugs. IUBMB Life *61*: 1013-1018, 2009.
5 Brewer GJ: Copper control as an antiangiogenic anticancer therapy: lessons from treating Wilson's disease. Exp Biol Med (Maywood) *226*: 665-673, 2001.
6 Lovejoy DB and Richardson DR: Iron chelators as anti-neoplastic agents: current developments and promise of the PIH class of chelators. Curr Med Chem *10*: 1035-1049, 2003.
7 Hatcher H, Planalp R, Cho J, Torti FM and Torti SV: Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci *65*: 1631-1652, 2008.
8 Sreejayan Rao MN: Curcuminoids as potent inhibitors of lipid peroxidation. J Pharm Pharmacol *46*: 1013-1016, 1994.
9 Jobin C, Bradham CA, Russo MP, Juma B, Narula AS, Brenner DA and Sartor RB: Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. J Immunol *163*: 3474-3483, 1999.
10 Singh S and Aggarwal BB: Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. J Biol Chem *270*: 24995-25000, 1995.
11 Beevers CS, Chen L, Liu L, Luo Y, Webster NJ and Huang S: Curcumin disrupts the Mammalian target of rapamycin-raptor complex. Cancer Res *69*: 1000-1008, 2009.
12 Bernabe-Pineda M, Ramirez-Silva MT, Romero-Romo MA, Gonzalez-Vergara E and Rojas-Hernandez A: Spectrophotometric and electrochemical determination of the formation constants of the complexes curcumin-Fe(III)-water and curcumin-Fe(II)-water. Spectrochim Acta A Mol Biomol Spectrosc *60*: 1105-1113, 2004.
13 Baum L and Ng A: Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. J Alzheimers Dis *6*: 367-377; discussion 443-449, 2004.
14 Ding WQ, Liu B, Vaught JL, Palmiter RD and Lind SE: Clioquinol and docosahexaenoic acid act synergistically to kill tumor cells. Mol Cancer Ther *5*: 1864-1872, 2006.
15 Ding WQ, Liu B, Vaught JL, Yamauchi H and Lind SE: Anticancer activity of the antibiotic clioquinol. Cancer Res *65*: 3389-3395, 2005.
16 Oikawa S, Yamada K, Yamashita N, Tada-Oikawa S and Kawanishi S: N-acetylcysteine, a cancer chemopreventive agent, causes oxidative damage to cellular and isolated DNA. Carcinogenesis *20*: 1485-1490, 1999.
17 Ding WQ, Vaught JL, Yamauchi H and Lind SE: Differential sensitivity of cancer cells to docosahexaenoic acid-induced cytotoxicity: the potential importance of down-regulation of superoxide dismutase 1 expression. Mol Cancer Ther *3*: 1109-1117, 2004.
18 Kunnumakkara AB, Guha S, Krishnan S, Diagaradjane P, Gelovani J and Aggarwal BB: Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. Cancer Res *67*: 3853-3861, 2007.
19 Barik A, Mishra B, Shen L, Mohan H, Kadam RM, Dutta S, Zhang HY and Priyadarsini KI: Evaluation of a new copper(II)-curcumin complex as superoxide dismutase mimic and its free radical reactions. Free Radic Biol Med *39*: 811-822, 2005.
20 Dancey J: mTOR signaling and drug development in cancer. Nat Rev Clin Oncol *7*: 209-219, 2010.
21 Pommier Y, Sordet O, Antony S, Hayward RL and Kohn KW: Apoptosis defects and chemotherapy resistance: molecular interaction maps and networks. Oncogene *23*: 2934-2949, 2004.
22 Zhai S, Yang L, Cui QC, Sun Y, Dou QP and Yan B: Tumor cellular proteasome inhibition and growth suppression by 8-hydroxyquinoline and clioquinol requires their capabilities to bind copper and transport copper into cells. J Biol Inorg Chem *15*: 259-269, 2010.
23 Chen D, Milacic V, Frezza M and Dou QP: Metal complexes, their cellular targets and potential for cancer therapy. Curr Pharm Des *15*: 777-791, 2009.

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