Curcumin Suppresses the Paclitaxel-Induced Nuclear Factor-κB in Breast Cancer Cells and Potentiates the Growth Inhibitory Effect of Paclitaxel in a Breast Cancer Nude Mice Model

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Abstract: Most anticancer agents activate nuclear factor kappa B (NF-κB), which can mediate cell survival, proliferation, and metastasis. Curcumin has been shown to inhibit the growth of various cancer cells, without toxicity to normal cells. The antitumor effects of curcumin could be due in part to the inactivation of NF-κB. We hypothesize that blocking NF-κB activity may augment paclitaxel cancer chemotherapy. In this study, we investigated whether the inactivation of NF-κB by curcumin would enhance the efficacy of paclitaxel for inhibiting breast cancer growth in vitro and in vivo. We confirmed that curcumin inhibited paclitaxel-induced activation of NF-κB and potentiated the growth inhibitory effect of paclitaxel in MDA-MB-231 breast cancer cells. The combination of curcumin with paclitaxel elicited significantly greater inhibition of cell growth and more apoptosis, compared with either agent alone. In an experimental breast cancer murine model using MDA-MB-231 cells, combination therapy with paclitaxel and curcumin significantly reduced tumor size and decreased tumor cell proliferation, increased apoptosis, and decreased the expression of matrix metalloprotease 9 compared with either agent alone. These results clearly suggest that a curcumin–paclitaxel combination could be a novel strategy for the treatment of breast cancer.

Key Words: breast cancer, curcumin, MDA-MB-231, nuclear factor-κB, paclitaxel

Cancer chemotherapeutic strategies should be devised to provide higher tumor response and lower toxicity. However, commonly used cytotoxic chemotherapy is largely associated with highly non-specific cytotoxicity, narrow therapeutic indices, and undesirable side effects. Paclitaxel is the drug of choice as it exhibits significant antitumor activity toward breast cancer among others (1). However, the success of paclitaxel chemotherapy in cancer patients is limited by myelotoxicity and neurotoxicity (2). Furthermore, tumors tend to acquire resistance to cytotoxic chemotherapeutic agents, including paclitaxel.

Although the molecular basis of resistance to paclitaxel is not well understood, mounting evidence supports the role of nuclear factor kappa B (NF-κB) in promoting cell survival and up-regulating genes important for tumor proliferation and metastasis (3), thereby affording protection against programmed cell death (4). It has been reported that other chemotherapeutic agents induced the activation of NF-κB in cancer cells, and this is believed to be responsible, at least in part, for drug resistance in cancer cells (5–7). Therefore, combining agents that inhibit NF-κB and induce apoptosis with chemotherapy may lead to a good tumor response with reduced systemic toxicity.

Curcumin, a yellow pigment present in the rhizome of tumeric, is one of the most extensively investigated phytochemicals with regard to chemopreventive properties and is considered pharmacologically safe. Curcumin has been shown to suppress NF-κB activation induced by various inflammatory stimuli (8), of which activation requires degradation of inhibitory factor of NF-κB alfa (IκBz) through the activation of inhibitory factor kappa B kinase (IKK) (9,10).

Based on the paclitaxel and curcumin data, we hypothesized that blocking NF-κB activity may
augment paclitaxel cancer chemotherapy. We tested this hypothesis by evaluating the effects of curcumin augmenting paclitaxel chemotherapy on cell growth, apoptosis, and metastatic potential using breast cancer cells and a nude mouse xenograft model.

**MATERIAL AND METHOD**

**Cell Line and Chemicals**

The human breast cancer cell line MDA-MB-231 was cultured in complete Eagle’s minimum essential medium. Paclitaxel (Sigma-Aldrich Chemical, St Louis, MO) and curcumin (Sigma-Aldrich Chemical) were dissolved in dimethylsulfoxide at appropriate concentrations.

**Mouse Xenograft Experiment**

Cells were injected into the mammary fat pad of 8-week-old athymic NCr-nu/nu mice (Dae-Han/Biolink, Daejeon, Korea). After 5 weeks of treatment with oral and injected water (control), daily oral curcumin (100 mg/kg), weekly IP injected paclitaxel (7 mg/kg), or curcumin plus paclitaxel (10 mice/group), the mice were killed and tumors in the mammary fat pad were harvested.

**Antibodies for Western Blot and Immunohistochemical Analysis**

Antibodies against inhibitory factor of NF-κB alfa (IκBα; Santa Cruz Biotechnology, Santa Cruz, CA) were used for Western blot analysis. Mouse anti-proliferating nuclear antigen (PCNA) and anti-matrix metalloprotease 9 (MMP-9; Santa Cruz Biotechnology) were used for immunohistochemical analysis.

**Growth Inhibitory and Apoptotic Assay**

Colorimetric dimethyl-thiazol-diphenyltetrazolium bromide proliferation assays were performed for growth inhibitory assay in cell culture by triplicate. 4,6-Diamidine-2, phenylindole dihydrochloride (DAPI) nuclear staining method was used for apoptosis in cell culture. The TUNEL technique (11) was used for evaluation of apoptosis in mouse experiments.

**Reporter Gene Assay**

Construction of the NF-κB-luciferase and inhibitory factor kappa B kinase/dominant negative (IKKβ/DN) is described elsewhere (12,13). The luciferase activity was determined using the Luciferase Assay System (Promega, Madison, WI).

**Statistical Analysis**

Data were analyzed using the unpaired two-tailed Student’s t-test. p < 0.05 was considered significant.

**RESULTS**

**Effects of Curcumin and Paclitaxel on MDA-MB-231 Cells In Vitro**

Curcumin and paclitaxel inhibited the growth of MDA-MB-231 cells with time and dose dependent manners (Fig. 1).

**Curcumin Inhibits Paclitaxel-Induced Degradation of IκBα**

NF-κB activation by most agents is known to require the phosphorylation and subsequent degradation of IκBα, an inhibitor of NF-κB. To confirm whether paclitaxel induces NF-κB activation by the degradation of IκBα, Western blot analysis of IκBα was performed. Cells were treated with paclitaxel (10 μM), curcumin (10 μM), or paclitaxel (10 μM) following 2 h pretreatment with curcumin (10 μM). Paclitaxel induced IκBα degradation in a time-dependent manner, whereas curcumin alone did not induce IκBα degradation (data not shown). However, paclitaxel following pretreatment with curcumin did not induce IκBα degradation (Fig. 2).

**Curcumin Inhibits Paclitaxel-Induced Activation of NF-κB and Potentiates the Growth Inhibitory Effect of Paclitaxel**

After treatment with curcumin and paclitaxel, alone and in combination, both curcumin and paclitaxel alone inhibited the growth of cells but the combination of paclitaxel with curcumin was more effective than either agent alone (Fig. 3b). Reporter gene assays showed a robust increase in NF-κB activation in cells treated with paclitaxel (Fig. 3a). Pretreatment with curcumin, or transfection with IKKβ/DN, blocked the drug-induced increase in reporter gene activation. The modulation by curcumin of paclitaxel-induced NF-κB activity and growth inhibition showed similar patterns of dose and time dependence in both assays. However, while IKKβ/DN transfection inhibited NF-κB activation more effectively than curcumin, the effect on cell
growth inhibition with paclitaxel did not exceed the effects of curcumin (Fig. 3a,b).

Curcumin Potentiates the Apoptotic Effects of Paclitaxel

Cells treated with curcumin (10 \( \mu \text{M} \)) and paclitaxel (10 \( \mu \text{M} \)) alone and in combination were then evaluated for apoptosis with DAPI staining. The combination of curcumin and paclitaxel increased tumor cell death more than either one alone or the control (Fig. 4).

Combination Therapy with Paclitaxel and Curcumin Significantly Inhibited Tumor Growth and Decreased Tumor Cell Proliferation Rate, MMP-9 Expression and Increased Apoptosis in the Nude Mouse Xenograft Model

Treatment with curcumin or paclitaxel alone modestly inhibited tumor growth compared with tumor growth in the control animals. However the curcumin and paclitaxel combination significantly inhibited tumor growth more than either agent alone (Table 1). Significantly fewer PCNA-positive cells and greater apoptotic fraction were found than did tumors from control or single-agent-treated mice. MMP-9 expression, reported to contribute to tumor progression, angiogenesis, and invasion (14,15) were very strong positive in the control group, strong positive in the curcumin-treated, moderately positive in the paclitaxel-treated and weakly positive in the combined curcumin and paclitaxel-treated group (Fig. 5).

**DISCUSSION**

We observed that curcumin inhibited the growth of MDA-MB-231 breast cancer cells in vitro in a dose and time-dependent manner, which is in agreement with other reports. Curcumin possesses antiproliferative activities against tumor cells (16), and inhibits tumor promotion in skin, oral, intestinal, and colon carcinogenesis models (17–19). Previous reports have
shown that curcumin suppresses a number of key elements in cellular signal transduction pathways including NF-κB (20,21), c-JUN/AP-1 activation (22), and phosphorylation reactions catalyzed by protein kinases (23). It has been reported that many chemotherapeutic agents induce activation of NF-κB in cancer cells and this is believed to be responsible in part for drug resistance in cancer cells (5–7). In this study we found that paclitaxel induced NF-κB activation in MDA-MB-231 cells, whereas curcumin alone did not. Furthermore, in curcumin-pretreated cells paclitaxel did not induce IκBα degradation, and NF-κB activation induced by paclitaxel was abrogated by curcumin treatment. Our study indicates that paclitaxel activates

Figure 3. Curcumin inhibits NF-κB activity induced by paclitaxel and potentiates the growth inhibitory effect of paclitaxel in MDA-MB-231 cells. The cells (5 × 10^3 cells/well) were incubated with paclitaxel alone (10 μM) or combined with curcumin (0.4–10 μM) and transfected with IKKβ DN for 24, 48, 72 h as indicated. (a) Curcumin, or transfection with a IKKβ DN, blocked the paclitaxel-induced NF-κB promoter-dependent luciferase activity. (b) Curcumin, or transfection with IKKβ DN, potentiates the growth inhibitory effect of paclitaxel. *p < 0.01 compared with paclitaxel alone, †p < 0.05, ‡p < 0.01 compared with IKKβ DN transfection.
Figure 4. Curcumin potentiates the apoptotic effects of paclitaxel in MDA-MB-231 cells. MDA-MB-231 cells were treated with curcumin (10 μM) or paclitaxel (10 μM) or the combination of the agents. The condensed chromosomes are seen as spots in the nucleus by DAPI staining; multinuclear cells are shown with arrowheads. Apoptotic cells were morphologically defined by cytoplasmic and nuclear shrinkage and chromatin condensation or fragmentation. C, control; Cur, curcumin; P, paclitaxel.

Table 1. Combination Therapy with Paclitaxel and Curcumin in a Nude Mouse Xenograft Model

| Group      | Tumor diameter (cm) | PCNA-positive cells | TUNEL-positive cells | MMP-9-positive cells |
|------------|---------------------|---------------------|----------------------|----------------------|
| Control    | 1.45 ± 0.53         | 56.27 ± 7.92        | 10.27 ± 3.46         | ++++                 |
| Curcumin   | 1.27 ± 0.79         | 36.30 ± 6.83        | 15.30 ± 2.63         | +++                  |
| Paclitaxel | 1.33 ± 0.51         | 50.49 ± 5.78        | 12.8 ± 3.02          | ++                   |
| Cur + P    | 0.32 ± 0.64*        | 0.67 ± 5.96         | 39.92 ± 3.48         | +                    |

Proliferating nuclear antigen and TUNEL-positive cells were counted in five random fields at ×200 magnification. Expression of matrix metalloprotease 9: ++++ (very strong positive), +++ (strong positive), ++ (moderate positive), + (weak positive). Cur, curcumin; P, paclitaxel.

*p < 0.001, /C160 p < 0.0001, /C224 p < 0.0002.

Figure 5. Effect of combination therapy with paclitaxel and curcumin in a nude mouse xenograft model. Proliferating nuclear antigen to evaluate tumor cell proliferation (×200) and TUNEL to evaluate apoptosis induction (×200). Matrix metalloprotease 9 to evaluate tumor cell invasive and metastatic potential (×200).
NF-κB in MDA-MB-231 breast cancer cells through 1kBz degradation.

The growth inhibitory effect from combining paclitaxel with curcumin was more effective than either agent alone, and curcumin potentiates the apoptotic effects of paclitaxel on MDA-MB-231 cells. This result supports the conclusion that one mechanism of the combined effect of paclitaxel and curcumin in growth inhibition on MDA-MB-231 breast cancer cells is through the inhibition of NF-κB activation. Our results showed that IKKβ/DN inhibited NF-κB activation more potently than curcumin in reporter gene assays, yet the effect on growth inhibition with paclitaxel did not exceed that of curcumin with paclitaxel. Accordingly, we suggest that other mechanisms by which curcumin enhance the efficacy of paclitaxel may exist, in addition to NF-κB inhibition. The combined effects of curcumin and paclitaxel may be partly due to the action of curcumin on tubulin polymerization. It is well known that paclitaxel exerts its growth inhibitory effect by binding tubulin and stabilizing microtubular structures, and this is thought to be the major mechanism of action of paclitaxel. However, Holy (24) has reported that disruption of mitotic spindle structure and induction of apoptosis occurred in cells treated with curcumin, albeit at a relatively high concentration. (25 μM). As in the in vitro studies, in the mouse xenograft model we found that the curcumin and paclitaxel combination treatment significantly inhibited tumor growth more than the control or either one alone. It should be noted that the 7 mg/kg IP paclitaxel dose used was lower than doses previously shown to be effective against breast cancer in the mouse xenograft model (25). Using this relatively less effective dose, we showed that the addition of curcumin resulted in a growth inhibitory effect that was as effective as higher, potentially toxic, doses of the chemotherapeutic drug. Examination of the histologic sections confirmed a reduced tumor proliferation rate by PCNA, and reduced expression of MMP-9, and showed a greater apoptotic fraction of tumor cells by TUNEL assay, in tumors from mice receiving the combined agents. NF-κB activation has been implicated in cell cycle control and metastasis. The expression of several genes, such as cyclin D1, MMP-9, COX2 and anti-apoptotic proteins, involved in tumor proliferation and metastasis are regulated by NF-κB (3,26). Our results are in agreement with previous reports that curcumin inhibits tumor proliferation, MMP-9 expression, and induces apoptosis (26–28).

In summary, combination therapy with paclitaxel and curcumin inhibits the proliferation of and induces apoptosis in MDA-MB-231 breast cancer cells in vitro and inhibits proliferation, MMP-9 expression, and induces apoptosis in the mouse xenograft model. We believe these preclinical data may provide an effective, novel approach for paclitaxel chemotherapy, with less toxicity, in breast cancer patients.

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