Full Paper

Obovatol Enhances Docetaxel-Induced Prostate and Colon Cancer Cell Death Through Inactivation of Nuclear Transcription Factor-κB

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Abstract. Nuclear transcription factor-κB (NF-κB) is constitutively activated in prostate and colon cancers and is related with the resistance of cancer cells against chemotherapeutics. Previously, we found that obovatol, an active compound isolated from Magnolia obovata, inhibited cancer cell growth through inhibition of NF-κB activity. We investigated here whether obovatol could sensitize cancer cells against docetaxel through inhibition of NF-κB activity in prostate cancer (LNCaP and PC-3) and colon cancer (SW620 and HCT116) cells. The combination treatment with each drug at one half the respective IC50 dose (5 μM obovatol + 5 nM docetaxel) was more effective and significant (60% – 70%) in the inhibition of cancer cell growth than single treatment by each drug (20% – 40%); inhibition was exerted through a significant increase of apoptosis induction (60% – 80%) by the combination treatment compared to the single treatment (10% – 30%). Correlating well with the synergistic inhibition (combination indices are less than 1 in all cell types), the combination significantly inhibited NF-κB activities as well as expression of NF-κB target apoptotic cell death proteins, but decreased anti-apoptotic cell death proteins. Similar combination effects of obovatol with other chemotherapeutic agents (paclitaxel, cisplatin, and doxorubicin) on the inhibition of cell growth and NF-κB activity were also found. These results indicate that obovatol augments cell growth inhibition by chemotherapeutics through inactivation of NF-κB and suggest that obovatol may have therapeutic advantages in the combination treatment with other chemotherapeutics.

[Supplementary Figure: available only at http://dx.doi.org/10.1254/jphs.09048FP]

Keywords: obovatol, docetaxel, combination treatment, nuclear transcription factor-κB (NF-κB), apoptotic cell death

Introduction

Nuclear transcription factor-κB (NF-κB) mediates tumor promotion, angiogenesis, metastasis, and chemo-therapeutic resistance through the expression of genes participating in malignant conversion and tumor promotion (1 – 3). In primary prostate cancer tissue samples, constitutive NF-κB activity has been suggested to have prognostic importance for a subset of primary tumors (4). Moreover, NF-κB was shown to be constitutively activated in human androgen-dependent prostate cancer cell lines (5). NF-κB has been also shown to be constitutively active in colon cancer cell lines and human tumor samples as well as nuclear of stromal macrophages in sporadic adenomatous polyps (6 – 8). It has been reported that intrinsically or constitutively activated NF-κB may be critical in the development of drug resistance in cancer cells (9 – 12). Therefore, several agents that are able to inhibit NF-κB function might be considered as a combination therapy with the conventional chemotherapeutics for lung cancer (11), prostate cancer (12), pancreatic cancer (13), breast cancer (14), and colon cancer (15).

Docetaxel, a semisynthetic taxoid produced from the needles of the European yew (Taxus baccata) tree, is an anti-neoplastic drug, belonging to the same class as...
paclitaxel. This anti-cancer drug is one of the most important new and active chemotherapeutic agents developed in recent years and has potential activity against human solid tumors including prostate and colon cancers that are refractory to conventional anti-cancer agents (16). Docetaxel has been shown to possess significant cell killing activity in a variety of tumor cells through induction of apoptosis. However, the use of high-dose docetaxel always induced toxic reactions, and significant toxicity has precluded the use of docetaxel as a monotherapy for cancer (17 – 19). Since low or moderate doses of docetaxel have no significant anti-tumor activity in patients (20 – 22), ways to reduce the dose of docetaxel without affecting its anti-tumor activity should be examined. Combination therapy is treatment using more than one anti-cancer drug and the use of two or more modes of treatment. Alternately or together, combination treatment to achieve optimum results against cancer has been demonstrated. The combination of protein bound polysaccharide (PSK) with docetaxel increased cytotoxicity towards pancreatic cancer cells by induction of apoptosis. Moreover, the enhanced apoptosis was because of the action of PSK in suppressing NF-κB activation (13). The combination of BAY 11-7085 and paclitaxel also increased the cytotoxic efficacy of paclitaxel in both in vitro and in vivo ovarian cancer models through inactivation of NF-κB (23).

The bark of obovata is widely used as a folk remedy for gastrointestinal disorders, anxiety, and cancer. It has reported that magnolol, honokiol, and obovatol are isolated from Magnolia obovata (24, 25). In a number of cancer cells, honokiol has been shown to have synergistic anti-tumor activity with other conventional chemotherapeutics, and the down regulation of NF-κB target gene expression is important for increase of susceptibility of cancer cell to chemotherapeutics (26, 27). The effect of honokiol and the combined effect with docetaxel on prostate cancer growth and bone metastasis were also reported (28). We previously also found that obovatol inhibits NF-κB in macrophages (29) and has inhibitory effect on colon and prostate cancer cell through inactivation of NF-κB and target gene expression (30). In the present study, we examined the combination effect of obovatol with docetaxel and other chemotherapeutic agents on cancer cell growth and induction of apoptosis in prostate and colon cancer cells through argumentation of NF-κB inactivation.

Materials and Methods

Materials

Obovatol (≥95% purity) (Fig. 1A) was isolated from the leaves of Magnolia obovata. The leaves of Magnolia obovata were harvested in the fall in Taejeon, Korea and identified as described elsewhere (30). Docetaxel was obtained from Samyang Genex Corporation (Daejeon, Korea). Docetaxel was produced by the procedure of semi-synthesis and purified. Briefly, crude docetaxel was obtained from semi-synthesis by the reaction of dehydroxybaccatin III with (3R,4S)-1-t-boc-3-triethylsilyloxy-4-phenylazetidin-2-one and then purified by recrystallization with MeOH/DW (methanol/distilled water) solution. The structure of docetaxel is shown in Fig. 1B. Docetaxel was dissolved in 0.01% DMSO.

Cell culture

Prostate (LNCaP and PC-3) and colon cancer (HCT116 and SW620) cells were obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco’s Modified Eagle Medium (DMEM), penicillin, streptomycin, and fetal bovine serum were purchased from Gibco Life Technologies (Grand Island, NY, USA).

Fig. 1. Structure of obovatol (A) and docetaxel (B).
Prostate and colon cancer cells were grown in RPMI1640 medium with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO₂ humidified air.

Cell viability assay
Prostate and colon cancer cells were plated at a density of 10⁵ cells/well in 96-well plates. The cytotoxic effect was evaluated in cells cultured for 24 and 48 h using the cell counting assay kit-8 (CCK-8) according to the manufacturer’s instructions (DOJINDO Laboratories, Kumamoto). Briefly, 10 μl of the CCK-8 solution was added to cells cultured for the designed time. The plates were incubated for 1–4 h. CCK-8 solution is reduced by dehydrogenases in the cells to give an orange-colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye (450 nm absorbance) generated by dehydrogenases in the cells is directly proportional to the number of living cells. Each assay was carried out in triplicate.

Western blot analysis
Cultured cells were washed twice with 1 × PBS, followed by the addition of 1 ml of PBS, and the cells were scraped into a cold Eppendorf tube. Cells were homogenized with lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.2% SDS, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml apro tin, 1% igapel 630 (Sigma Chem. Co., St. Louis, MO, USA), 10 mM NaF, 0.5 mM EDTA, 0.1 mM EGTA, and 0.5% sodium deoxycholate] and centrifuged at 15,000 × g for 10 min followed by the addition of 1 ml (50,000 – 200,000 cpm) of [γ-P³²]ATP-labeled oligonucleotide and another 20 min of incubation at room temperature. Subsequently 1 μl of gel loading buffer was added to each reaction and loaded onto a 4% nondenaturing gel and then transferred to a nitrocellulose membrane (Hybond ECL; Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Blots were blocked for 2 h at room temperature with 5% (w/v) non-fat dried milk in Tris-buffered saline [10 mM Tris (pH 8.0) and 150 mM NaCl] solution containing 0.05% Tween-20. The membranes were immunoblotted with the following primary specific antibodies: rabbit polyclonal for Bax (1:500 dilution; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and caspase-3, cleaved caspase-3, caspase-9, cleaved caspase-9, poly ADP ribose polymerase (PARP), cleaved PARP, Bcl-2, X inhibitor of apoptosis protein (XIAP), cellular-inhibitor of apoptosis protein 1 (c-IAP1) (1:1000 dilution, Cell Signaling Technology, Inc., Beverly, MA, USA). The blot was then incubated with the corresponding conjugated anti-rabbit or anti-mouse immunoglobulin G-horseradish peroxidase (1:2000 dilution, Santa Cruz Biotechnology Inc.). Immunoreactive proteins were detected with the ECL Western blotting detection system. The relative density of the protein bands was scanned by densitometry using MyImage (SLB, Seoul, Korea) and quantified by Labworks 4.0 software (UVP Inc., Upland, CA, USA).

Gel electro mobility shift assay
Gel shift assays were performed according to the manufacturer’s recommendations (Promega, Madison, WI, USA). Briefly, 2 × 10⁶ cells/ml was washed twice with 1 × PBS, followed by the addition of 1 ml of PBS, and the cells were scraped into a cold Eppendorf tube. Cells were spun down at 15,000 × g for 1 min, and the resulting supernatant was removed. Solution A (50 mM HEPES, pH 7.4, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1 μg/ml PMSF, 1 μg/ml peptatin A, 1 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml apr tin, and 0.5% Nonidet P-40) was added to the pellet in a 2:1 ratio (v/v) and allowed to incubate on ice for 10 min. Solution C (solution A + 10% glycerol and 400 mM KCl) was added to the pellet in a 2:1 ratio (v/v) and vortexed on ice for 20 min. The cells were centrifuged at 15,000 × g for 7 min, and the resulting nuclear-extract supernatant was collected in a chilled Eppendorf tube. Consensus oligonucleotides were end-labeled using T4 polynucleotide kinase and [γ-P³²]ATP for 10 min at 37°C. Gel shift reactions were assembled and allowed to incubate at room temperature for 10 min followed by the addition of 1 μl (50,000 – 200,000 cpm) of [γ-P³²]ATP-labeled oligonucleotide and another 20 min of incubation at room temperature. Subsequently 1 μl of gel loading buffer was added to each reaction and loaded onto a 4% non-denaturing gel and electrophoresis continued until the dye was three-fourths of the way down the gel. In electrophoretic mobility shift assay (EMSA) competition studies, a 100-fold excess of unlabeled competitor oligonucleotide NF-κB was incubated with nuclear extract for 30 min before the addition of the labeled probe. The mixture with the labeled probe was incubated for another 30 min on ice. For the supershift assay, 0.5 μg of the indicated antibodies (p65 and p50) were further added and the mixture was incubated for an additional 30 min on ice and then subjected to gel electrophoresis using 6% native polyacrylamide gels in 1 × Tris-borate-EDTA buffer for 2 h. The gel was dried at 80°C for 1 h and exposed to film overnight at −70°C. The relative density of the protein bands was scanned by densitometry using MyImage and quantified by Labworks 4.0 software.

Transfection and assay of luciferase activity
Prostate and colon cancer cells (2.5 × 10⁵ cells/cm²)
were plated in 24-well plates and transiently transfected with pNF-κB-Luc plasmid (5 × NF-κB; Stratagene, CA, USA) using a mixture of plasmid and lipofect AMINE PLUS in OPTI-MEN according to manufacturer’s specifications (Invitrogen, Carlsbad, CA, USA). The transfected cells were incubated with 400 μl of fresh medium containing 5 μM obovatol and 5 nM docetaxel for 6 h. Each value is the mean ± S.D. of three independent experiments performed in triplicate. RLU is relative to luciferase activity in unstimulated cells. *P<0.05 indicates statistically significant differences from the control group. B: Nuclear extract from prostate and colon cancer cells treated with 1, 5, 10, 20, and 50 nM docetaxel for 1 h was used in the binding reactions of 32P-end-labeled oligonucleotide containing the κB sequence. The activation of NF-κB was investigated using EMSA as described in Materials and Methods. C: Nuclear extract from prostate and colon cancer cells co-treated with 5 μM obovatol and 5 nM docetaxel for 1 h was used in the binding reactions of 32P-end-labeled oligonucleotide containing the κB sequence. Quantification of band intensities from three independent experimental results was performed by densitometry using MyImage (SLB) and the value under each band indicated as fold difference from the untreated control group.

Fig. 2. Effect of the combination treatment of obovatol and docetaxel on the NF-κB transcriptional (A) and DNA binding activity (B and C) in prostate and colon cancer cells. A: Prostate and colon cancer cells were transfected with pNF-κB-Luc plasmid (5 × NF-κB) for 4 h. The transfected cells were incubated with 400 μl of fresh medium containing 5 μM obovatol and 5 nM docetaxel for 6 h. Each value is the mean ± S.D. of three independent experiments performed in triplicate. RLU is relative to luciferase activity in unstimulated cells. *P<0.05 indicates statistically significant differences from the control group. B: Nuclear extract from prostate and colon cancer cells treated with 1, 5, 10, 20, and 50 nM docetaxel for 1 h was used in the binding reactions of 32P-end-labeled oligonucleotide containing the κB sequence. The activation of NF-κB was investigated using EMSA as described in Materials and Methods. C: Nuclear extract from prostate and colon cancer cells co-treated with 5 μM obovatol and 5 nM docetaxel for 1 h was used in the binding reactions of 32P-end-labeled oligonucleotide containing the κB sequence. Quantification of band intensities from three independent experimental results was performed by densitometry using MyImage (SLB) and the value under each band indicated as fold difference from the untreated control group.

Detection of apoptosis
Prostate and colon cancer cells (2.5 × 10⁵ cells/cm²) were cultured on a chamber slide (Lab-Tak II chamber slider system; Nalge Nunc Int., Naperville, IL, USA), fixed in 4% paraformaldehyde, and membrane-permeabilized by exposure for 30 min to 0.1% Triton X-100 in
PBS at room temperature. Terminal dUTP nick-end labeling to detect apoptotic cells (TUNEL) assays were performed by using the in situ Cell Death Detection Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer’s instructions. For 4′,6-diamidino-2-phenylindole (DAPI) staining, slides were incubated for 30 min at room temperature in the dark with mounting medium for fluorescence containing DAPI (Vector Laboratories, Inc., Burlingame, CA, USA). The cells were then observed through a fluorescence microscope (Leica Microsystems AG, Wetzlar, Germany). Total number of cells in a given area was determined by using DAPI nuclear staining. The apoptotic index was determined as the number of DAPI-stained TUNEL-positive stained cells divided by the total cell number counted ×100.

Statistical analysis

Data were analyzed by one-way analysis of variance followed by Dunnett’s test as a post hoc test. Differences were considered significant at the P<0.05 level. The combination index (CI) was calculated by the methods described by Chou (31).

Results

Effect of the combination of obovatol and docetaxel on the NF-κB activation

We were interested in whether obovatol inhibits prostate and colon cancer cell growth by inactivation of NF-κB because activation of NF-κB is a critical factor for cancer cell survival and chemotherapeutic resistance. To determine the effect of the combination of obovatol and docetaxel on the NF-κB–dependent transcription activity, we transiently transfected the cells with NF-κB–regulated luciferase reporter construct, and then the transfected cells were stimulated with tumor necrosis factor-α (TNF-α) (5 ng/ml) alone to activate transcriptional activity of NF-κB. Our previous data (25) and preliminary data showed that the IC₅₀ value for inhibition of cell growth by obovatol is 18–23 μM, whereas docetaxel has an IC₅₀ of 10 nM. Thus, for each drug, we used a dose corresponding to half the IC₅₀ dose in the combination treatment. The combination treatment of obovatol (5 μM) and docetaxel (5 nM) more significantly inhibited TNF-α–induced transcriptional activation of NF-κB in prostate and colon cancer cell lines than either single agent alone (Fig. 2A). We next investigated of the combination of docetaxel and obovatol on the
Constitutive activation of NF-κB was observed in prostate and colon cancer cells, and the treatment with docetaxel did not change or slightly increased the NF-κB activity by 5 nM docetaxel in LNCaP, PC-3, HCT116, and SW620 cells (Fig. 2B). However, similar to the previous finding (30), the constitutively activated NF-κB was decreased by the obovatol alone at more than 10 μM (data not shown). In the combination study, 5 μM obovatol and 5 nM docetaxel resulted in a strong synergistic inhibitory effect on the DNA binding activity of NF-κB, whereas treatment with either docetaxel or obovatol alone treatment did not or only slightly inhibited NF-κB (Fig. 2C). Consistent with the inhibition of the transcriptional activity, the inhibitory combination effect was also found in the presence of TNF-α (Fig. 3A). The DNA binding activity was confirmed by the competition assay as well as supershift assay using an excess amount of unlabelled NF-κB and the specific antibodies for NF-κB subunit p65 and p50 (Fig. 3B).

**Obovatol enhances prostate and colon cancer cells growth inhibition by docetaxel**

To investigate the inhibitory effect of the combination of obovatol with docetaxel on the prostate and colon cancer cell growth, we analyzed cell growth by a CCK-8 assay kit. The effect of docetaxel alone on the prostate and colon cancer cell growth was determined. Our data showed a significant concentration-dependent inhibition of cell viability by docetaxel in prostate and colon cancer cells (data not shown). Subsequent studies were undertaken to examine whether the prostate and colon cancer cells were more sensitive to growth inhibition by the combination of obovatol with docetaxel. The addition of both agents together for 24 or 48 h resulted in a strong synergistic inhibitory effect on cell growth, showing...
50% or 70% (LNCaP), 70% or 90% (PC-3), 40% or 55% (HCT116), and 40% or 60% (SW620) inhibition, whereas treatment with either obovatol (5 μM) or docetaxel (5 nM) alone caused a 10% – 30% inhibition (40% or 60% inhibition in PC-3 cells) in both prostate and colon cancer cell growth (Fig. 4). The CI value was 0.349 in LNCaP cells, 0.283 in PC-3 cells, 0.717 in HCT116 cells, and 0.610 in SW620 cells.

Effect of the combination of obovatol and docetaxel on the apoptotic cell death

Cell death contributed to the cell growth inhibition, so we evaluated changes in the apoptotic cell death in prostate and colon cancer cells using DAPI and TUNEL staining analyzed by fluorescence microscopy. Apoptotic cell number (DAPI-stained TUNEL-positive cells) of prostate and colon cancer cells was slightly increased by treatment with obovatol or docetaxel alone (10% – 30%). However, the combination treatment resulted in about 60% – 80% induction of apoptotic cell death in both prostate and colon cancer cells (Fig. 5).

Obvatol augments docetaxel-induced the expression of apoptotic regulatory proteins

To identify the mechanism for the enhanced apoptotic response by obovatol and docetaxel combination treatment, we next assessed the expression of pro and anti-apoptotic proteins by Western blotting. Cells were treated with obovatol (5 μM) or docetaxel (5 nM) alone or with the combination of obovatol and docetaxel for
24 h, and the whole-cell extract was subjected to Western blotting. Our data showed that the combination treatment substantially inhibited the levels of all tested marker proteins that favor cell survival such as Bcl-2, XIAP, and c-IAP1. However, the expression of pro-apoptotic proteins, Bax and active forms of caspases-3 (cleaved caspase-3) and caspase-9 (cleaved caspase-9), was more significantly increased by the combination treatment of obovatol and docetaxel compared to the expression in the obovatol or docetaxel alone treated cells (Fig. 6).

**Effect of the combination of obovatol with other chemotherapeutics on the cancer cell growth**

To generalize the combination effect of obovatol with other chemotherapeutics, we investigated the cancer cell growth inhibitory effect of the combination of obovatol with other chemotherapeutics in prostate and colon cancer cells. For these studies, cells were treated with obovatol (5 μM) alone or in combination with one of the following drugs at a concentration equal to half the respective IC_{50} dose: paclitaxel (10 nM), cisplatin (10 μM), and doxorubicin (2 μM) (Fig. 7A), which are the most used chemotherapeutics. We found that the combination treatment of cells with both obovatol and other chemotherapeutics for 24 and 48 h caused a significant growth inhibition (60% – 80% inhibition) in prostate (PC-3) and colon (SW620) cancer cells compared with growth inhibition by single treatment of each agent (20% – 30% inhibition) (Fig. 7B).

**Effect of the combination of obovatol with other chemotherapeutics on the NF-κB activity**

We investigated whether the combination of obovatol with other chemotherapeutics inhibits prostate and colon cancer cell growth by inactivation of NF-κB. Consistent with the effect of the drug combination on cancer cell growth inhibition, the combination of obovatol with other chemotherapeutics showed a greater decrease in the NF-κB DNA–binding activity compared with those by single chemotherapeutic treatment in both prostate (PC-3) and colon (SW620) cancer cells (Fig. 8).

**Discussion**

The findings of present study are that obovatol synergizes or additively argues the therapeutic effect of docetaxel and other therapeutic agents in prostate and colon cancer cells by growth inhibition and induction of apoptosis through inactivation of NF-κB. Conventional cancer therapies, including surgery, chemotherapy, and radiotherapy, as single modalities have a limit because of the lower efficacy and high toxicity. Thus, the strategies of cancer treatment using combined therapies or combined agents are considered more promising for higher efficacy, resulting in better survival. The combination treatment may also decrease the systemic toxicity because lower doses could be used.

Several reports have demonstrated the advantage of combination treatments of chemotherapeutics. Genistein
potentiated growth inhibition and apoptotic cell death caused by docetaxel, cisplatin, doxorubicin, 5-fluorouracil (5-FU), and gemcitabine in prostate, breast, pancreas, colon, and lung cancers (33–36). Curcumin and celecoxib combination synergistically increased colon cancer cell growth inhibition (37–39). Even though the advantage of combination treatment in cancer cell growth control has been demonstrated, the possible targets of combination treatment are not clear. NF-κB has been implicated in both carcinogenesis and the development of drug resistance in cancer cells (40–42). While activation of NF-κB may induce apoptosis in certain situations (43–46), most reports suggest that intrinsically or constitutively activated NF-κB may be critical in the development of drug resistance and survival signals that counteract apoptosis in cancer cells (47–52). Prevention of NF-κB activation, therefore, may represent a promising opportunity for the widening therapeutic windows in translational cancer research (52–54). NF-κB is also the most reliable target of the combination chemotherapeutic treatment to overcome drug resistance. When curcumin was combined with cisplatin or doxorubicin, the cell susceptibility is increased, where the levels of NF-κB remained lower than those predicted from the effects of the single agents (38, 39). The combination of sulindac with arsenic trioxide also increased apoptotic cell death of HCT116 colon cancer cells through inactivation of NF-κB (55). Moreover, very similar to our finding, the anti-cancer effects of the combination of honokiol with other chemo-
therapeutics on breast, ovarian, and lung cancer cells were reported (56–60), and these anti-cancer effects were related to the inactivation of NF-κB (26, 27). We recently also found that the combination of docetaxel and thiacremonone, a sulfur compound isolated from garlic, showed a synergistic anti-cancer effect through inactivation of NF-κB (61). We also found that obovatol has the ability to inactivate NF-κB in macrophages as well as colon cancer cells (28, 30). These data suggest that NF-κB could be a critical target for the combination of obovatol and docetaxel, and the ability of blunting NF-κB by this combination might be significant for increasing the cell susceptibility.

Apoptosis is an important mechanism to eliminate unwanted cells, and deregulation of this process is implicated in the pathogenesis of cancer development. It is well known that NF-κB–mediated expression of Bcl-2, IAP1/2, and survivin protects cancer cells from apoptosis, whereas Bax, caspase-3, and caspase-9 inhibit cancer cell growth and induce apoptosis. Our previous studies showed that NF-κB inactivation–mediated decrease of anti-apoptotic genes, but increase of apoptotic genes, enhanced prostate and colon cancer cell growth inhibition in vitro and in vivo (12, 15, 61). The present data showed that the combination treatment increased expressions of apoptotic proteins like active caspase-3 and Bax, but decreased Bcl-2, IAP, and XIAP expression. Hence, an alteration in the levels of anti-apoptotic and pro-apoptotic proteins is likely to influence apoptosis. These present data suggest that a down-regulation of NF-κB–mediated anti-apoptotic genes and an up-regulation of apoptotic genes by the combination treatment are significant for sensitization of cancer cells to the combination of obovatol and docetaxel.
The inhibitory effects of the combination of obovatol and docetaxel against both prostate and colon cancer cell growth were synergistic (the CI values are less than 1 in all cells), so the present results suggest that this combination may be a promising regimen for the treatment of these cancers. However, the inhibitory effect of the combination against prostate cancer cell growth (CI values are 0.349 in LNCaP cells and 0.283 in PC-3 cells) is more potent than that in colon cancer (CI values are 0.717 in HCT116 cells and 0.610 in SW620 cells). At present, we do not know what mechanism causes the difference in the inhibitory effects between these two cell lines. However, cell type specificity in the combination effect on the cancer cell growth may be a possibility. In fact, the prostate cancer cell showed more susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone, and this greater susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone, and this greater susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone, and this greater susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone, and this greater susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone, and this greater susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone, and this greater susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone, and this greater susceptibility than colon cancer in the treatment with either docetaxel or obovatol alone.

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References


