The effects of combining docetaxel and cyclooxygenase-2 inhibitors on proliferation and apoptosis in epithelial ovarian cancer

Adnan R. Munkarah[a,b,d], Rouba Ali-Fehmi[a,c], John Z. Jiang[a,b], Eslam Elhammady[a], John M. Malone Jr[a,b,d] and Ghassan M. Saed[a,b]

In-vitro studies have shown that taxanes can upregulate cellular cyclooxygenase-2 expression. The purpose of this study is to evaluate the effects of the combination, cyclooxygenase-2 inhibitor and docetaxel, on epithelial ovarian cancer cells. Four epithelial ovarian cancer cell lines (MDAH-2774, SKOV3, OVCAR and CaOV-3) were treated with the specific cyclooxygenase-2 inhibitor NS398 (10 or 100 μmol/l) and docetaxel (0.1, 1 or 10 μmol/l) in various combinations. Apoptosis in the ovarian cancer cells was assessed using TUNEL assay. Multiplex reverse transcription-PCR was used to determine mRNA levels of cyclooxygenase-2, bcl-2 and bax. Treatment of all epithelial ovarian cancer cells with docetaxel resulted in significant apoptotic death. Concurrent treatment of MDAH-2774, SKOV3 and OVCAR cells with docetaxel and NS398 resulted in the reduction of the taxane-induced apoptosis. Similar reduction was seen when the cells were exposed to NS398 for 4 h before docetaxel treatment. Conversely, treating the MDAH-2774 and SKOV3 cells with docetaxel followed by NS398 resulted in a significant increase in apoptosis compared with treatment with the taxane alone. bax mRNA levels were significantly reduced in SKOV3 cells treated concurrently with NS398 and docetaxel; bcl-2 mRNA levels showed no change. When combining docetaxel and a cyclooxygenase-2 inhibitor in the treatment of ovarian cancer cells, the sequencing of the drugs seems to have an important influence on the overall outcome. Using the cyclooxygenase-2 inhibitor before or concurrently with the taxane will result in a reduction of cellular apoptotic death. This might be due to a reduction in the expression of the proapototic gene bax. *Anti-Cancer Drugs* 18:889–896 © 2007 Lippincott Williams & Wilkins.

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[a]Wayne State University School of Medicine, [b]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology and [c]Department of Pathology and [d]Karmanos Cancer Institute, Detroit, Michigan, USA

Correspondence to Dr Adnan R. Munkarah, MD, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Harper Professional Office Building, 4160 John R., Suite 2135, Detroit, MI 48201, USA
Tel: +1 313 745 0735; fax: +1 313 993 8857;
e-mail: amunkara@med.wayne.edu

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Introduction

Ovarian cancer continues to be among the leading causes of cancer deaths in women. In the past decade, a number of studies have shown that the addition of paclitaxel to platinum in the primary treatment of epithelial ovarian cancer (EOC) has resulted in prolonged survival [1]. Docetaxel is a taxane with a similar cellular activity to paclitaxel. It promotes microtubule assembly and stabilization, and inhibits tubulin disassembly [2]. It also induces apoptotic cell death through a bcl-2-associated mechanism [3]. In the preclinical setting, docetaxel seems to have a number of advantages over paclitaxel. First, it has a greater affinity for tubulin. Second, there is high intracellular uptake and slow efflux rates *in vitro* leading to longer intracellular retention half-life. Third, it promotes microtubule polymerization at lower levels. In addition, there is only partial cross-resistance between paclitaxel and docetaxel in paclitaxel-resistant cell lines. In recent trials of primary chemotherapy for ovarian cancer, the use of docetaxel instead of paclitaxel has resulted in at least equivalent response rates and patients' survival with diminished neurotoxicity [4].

Evidence from in-vitro and in-vivo studies suggest an important role for prostaglandins and their synthesizing enzyme cyclooxygenase-2 (COX-2) in carcinogenesis [5–8]. Prolonged intake of drugs which inhibit COX-2 reduces the incidence of a number of malignancies [9–11]. Laboratory data have also revealed that exposure of various human carcinoma cell lines to COX-2 inhibitors induces apoptotic cell death *in vitro* [12–14]. A number of investigators evaluated the effects of combining these drugs with various chemotherapeutic agents and showed that the combination significantly increases the cytotoxicity of a number of chemotherapy drugs [15–20]. We have previously reported that simultaneous treatment of EOC cell lines with paclitaxel and the specific COX-2 inhibitor, NS398, resulted in a paradoxical inhibition of the paclitaxel-induced apoptosis [21].

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On the basis of data from various investigators as well as our laboratory, we got interested in investigating the role of COX-2 inhibitors as an adjuvant treatment to chemotherapy in ovarian cancer. The goal of this study was to determine the effects of sequential treatment of a number of EOC cell lines with docetaxel and the specific COX-2 inhibitor NS398 on: (1) apoptosis and cell growth, (2) COX-2, bcl-2 and bax mRNA expression, and (3) cell cycle progression.

Methods

Culture of ovarian cancer cell lines

A total of four EOC cell lines were used for the different experiments of this study: SKOV3, MDAH-2774, OVCAR and CaOV-3.

The human EOC cell lines, SKOV3 and MDAH-2774, were grown in T-150 flasks with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/l L-glutamine, 100 U of penicillin, 0.1 µg of streptomycin and 0.25 µg of amphotericin B. The CaOV-3 and OVCAR cells were maintained in a humidified atmosphere in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 4 mmol/l of L-glutamine at 37.0°C/5% CO_2. Cells were maintained at confluency at 37°C incubation with 5% CO_2. The media were changed three times per week. For each experiment, cells were plated out in 0.3% FBS-containing medium in 100-cm^2 culture dishes at a cell density of approximately 1×10^6 cells per dish and incubated for another 48 h. Cell cultures were treated with increasing concentrations of docetaxel (0.1, 1 and 10 µmol/l), NS398 (10 and 100 µmol/l) and a combination of both drugs. The NS398 concentrations used in our experiments are relevant clinically because the doses of COX-2 inhibitors recommended to patients will achieve serum concentrations in the range of 1×10^{-4} to 2×10^{-5} mol/l. NS398 was solubilized in dimethylsulfoxide which was also added in the control cultures.

In-situ analysis of DNA integrity (TUNEL assay)

Ovarian cancer cells were grown in Lab-Tek chamber slides (Nunc, Naperville, Illinois, USA). Apoptosis was assessed using the TUNEL technique as described by the Promega Apoptosis Detection System (Promega; Madison, Wisconsin, USA) and in a previous publication [21]. Briefly, slides were treated with proteinase K (20 µg/ml) in 10 mmol/l Tris–HCl (pH 8.0) for 15 min at room temperature and washed four times for 2 min in ddH_2O. Endogenous peroxidase was inactivated by incubating the sections for 5 min in 3% H_2O_2 at room temperature and then washed three times in ddH_2O. Slides were preincubated for 10 min at room temperature in TdT buffer (30 mmol/l Tris–HCl–140 mmol/l Na cacodylate–1 mmol/l cobalt chloride) and incubated in a moisture chamber for 1 h at 37°C with 20–30 µ1 of TdT buffer with 0.5 U of TdT/µl and 40 µmol/l of fluorescein 12-dUTP. The reaction was stopped by transferring the slides to 2 × saline sodium citrate buffer (300 mmol/l NaCl–30 mmol/l sodium citrate) for 15 min at room temperature followed by the addition of propidium iodide to stain all cells. Fluorescein and propidium iodide were used to stain DNA and the DNA content of the cells was determined by flow cytometry. Detection of localized green fluorescence of apoptotic cells (fluorescein 12-dUTP) and in red background (propidium iodide) was performed by fluorescence microscopy. Quantitative analysis was performed by flow cytometric analysis of the changes in mean fluorescence for cell suspensions. Positive controls were performed by treating cells with DNase I (1 µg/ml) in TdT buffer for 10 min at room temperature before incubation with biotinylated nucleotide.

Cell proliferation measurement by flow cytometric analysis

The proliferative fraction, which consists of cells in the G_2/M and S phases, was determined using the cell flow cytometry (Wayne State University core facility), from ovarian cancer cell lines under all treatment conditions [23–25].

Cell growth and viability assay

The Trypan blue dye exclusion was used to determine the percentage of viable cells after the various treatments. Cell suspension was prepared at a 1 : 1 dilution with 0.4% Trypan blue solution. The counting chamber of a hemocytometer was loaded. The number of stained cells and the total number of cells were counted. The percentage of viable cells was determined by calculating the percentage of unstained cells.

Multiplex reverse transcription-PCR for cyclooxygenase-2, bcl-2 and bax

The multiplex reverse transcription (RT)-PCR technique was utilized to detect and compare mRNA levels of COX-2, bcl-2 and bax in ovarian cancer cells after various treatments.

Reverse transcription-PCR

RNA was isolated using the monophasic solution of phenol and GITC/Trizol method as previously described [21]. RNA was treated with DNase 1 RNase-free in 10 mmol/l Tris–C1, pH 8.3, 50 mmol/l of KCl, 1.5 mmol/l of $MgC1_2$ in the presence of RNasin ribonuclease inhibitor and incubated at 37°C for 30 min. For cDNA synthesis, 20 µg of RNA was heated to 68°C for 10 min in the presence of 2 µl oligo dT primer and then rapidly chilled on ice. A master mix containing 4 µl of 5 × first strand buffer, 2 µl of 0.1 mol/l dithiothreitol, 1 µl of 10 mmol/l deoxynucleoside triphosphate mix, 1 µl of superscript II (200 U; Life Technologies Inc.; Gaithersburg, Maryland, USA) and 1 µl of RNase inhibitor was

added, and each reaction was incubated at 42°C for 1 h. Aliquots from the cDNA reaction were then PCR-amplified in 100-µl reactions as follows: 10 µl of 10 × PCR reaction buffer, 1 mmol/l from each of the deoxynucleoside triphosphate, 20 µmol/l from each primers and 2.5 U Taq polymerase enzyme. Mineral oil was added to prevent evaporation. The reaction is initiated by heat denaturation at 95°C for 1 min, annealing the primers for 2 min at 60°C, and then extension for 3 min at 72°C. This was repeated for 35 cycles using the PCR (Perkin-Elmer, Waltham, Massachusetts, USA). After the final cycle the temperature is maintained at 72°C for 7 min to allow completion of synthesis of amplified products. Analysis of PCR-amplified products was performed by fractionation over a 2% agarose gel followed by ethidium bromide staining of DNA bands. Scanning densometry was used to determine the ratio of intensity of each band relative to β-actin, using NIH Image analysis program.

Primer design and controls

Optimal oligonucleotide primer pairs for multiplex RT-PCR amplification of oligo dT-primed reverse-transcribed cDNA were selected with the aid of the computer program Oligo 4.0 (National Biosciences, Plymouth, Minnesota, USA). The human oligonucleotide primers, which amplify variable portions of the protein coding regions, that were used are listed in Table 1.

Statistical analysis

The docetaxel and NS398 experiments were run in triplicates; the mean as well as standard deviations and standard errors were computed. The means were then compared using analysis of variance and Student's *t*-test. In viability assays, the log of the mean difference in number of viable cells (and before after treatment) was determined and comparisons were made using Student's *t*-test with significance set at $P < 0.05$.

Results

Effect of treatment with docetaxel and NS398 on apoptosis and cell growth

Sequential treatment of the EOC cells MDAH-2774, SKOV3 and OVCAR with NS398 (10, 100 µmol/l) for 4 h followed by docetaxel (10 µmol/l) for 24 h showed a significant inhibition of drug-induced apoptosis compared with cells treated with docetaxel alone (Fig. 1). Concurrent treatment of the cells with docetaxel and NS398 resulted in a similar reduction of docetaxel-induced cytotoxicity (Fig. 1). The CaOV-3 cells were less sensitive to docetaxel treatment at 24 h; pretreatment of those cells with NS398 did not result in a significant inhibition of apoptosis at that time point. The proliferative fraction of the cells was higher in the combined and sequential drug treatment compared to treatment with docetaxel alone (Fig. 1). Flow cytometry analysis revealed that either sequential or concurrent treatments resulted in apoptosis and sequestering in G_2 phase with no evidence of any significant G_0/G_1 arrest resulting from the use of the COX-2 inhibitor (Fig. 2).

The sequence of administration of drugs was then reversed. Cells were treated with docetaxel at 0.1 µmol/l for 24 h followed by NS398 100 µmol/l for 24 h (schedule 1) or 72 h (schedule 2). That treatment sequence had no negative effect on docetaxel-induced apoptosis for any of the cell lines. On the contrary, there was an increase in apoptotic death of the MDAH-2774 and SKOV3 cells that was more pronounced after 72 h of N-S398 treatment as compared with docetaxel treatment alone. For the CaOV-3, there was increased apoptosis with the combination treatment compared with docetaxel alone at 24 h (data not shown). For the OVCAR cells, the sequential treatment did not result in any significant change in apoptosis rate compared with docetaxel treatment alone (Fig. 3).

The Trypan blue assay was then used to assess cell growth and viability for the MDAH-2774 and SKOV3 cells in response to the different treatments. We confirmed that (1) docetaxel treatment resulted in a significant cancer cell death, and (2) the addition of NS398 for 72 h after docetaxel treatment results in an additional 16 and 29% reduction in the number of viable cells recovered at the end of treatment for MDAH-2774 and SKOV3 cells, respectively (Fig. 3e).

Effects docetaxel and NS398 treatment on bcl-2, bax and cyclooxygenase-2 mRNA levels

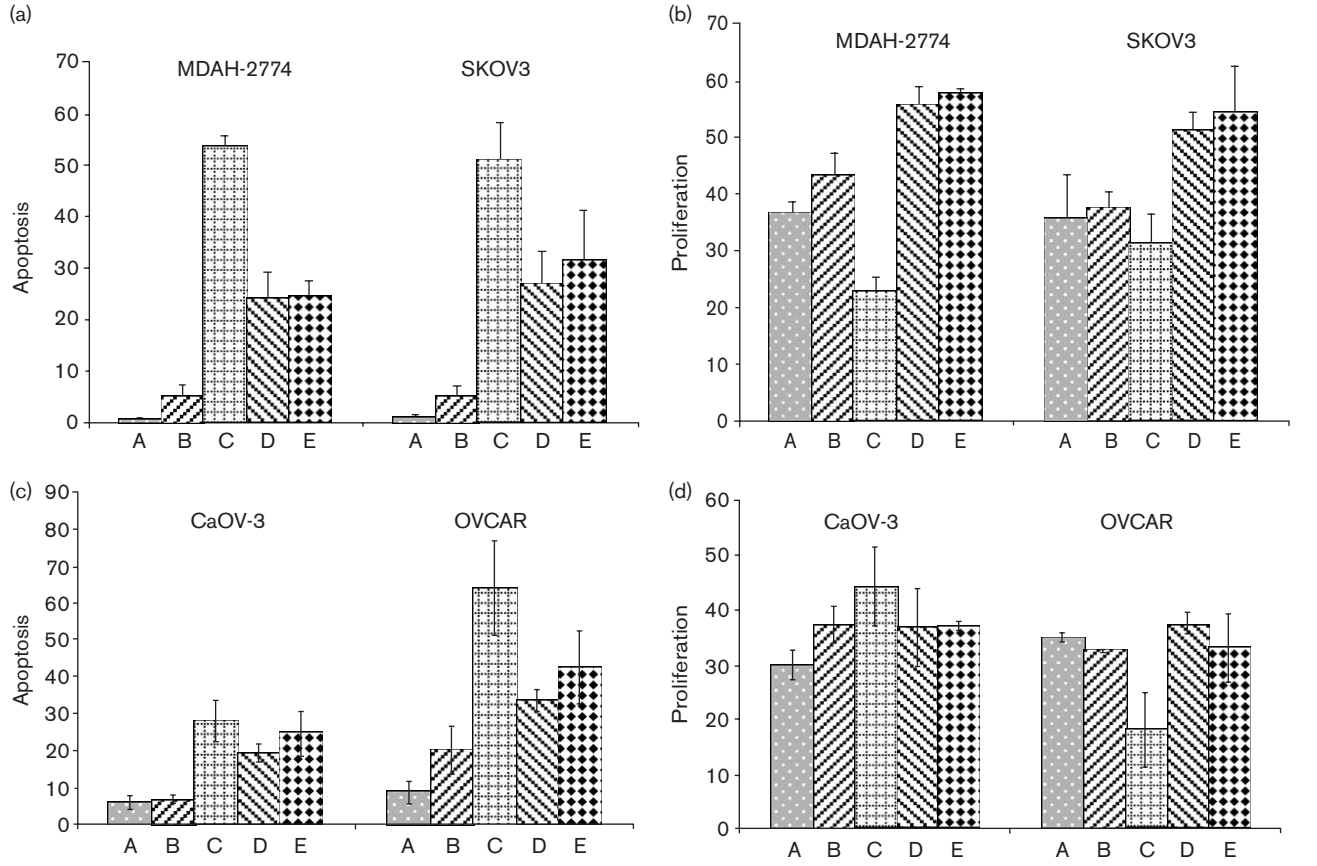
In an attempt to investigate the above treatment effects resulting from the various sequences of drug administration, the effect of the different treatment schedules on the levels of COX-2, bcl-2 and bax mRNA in SKOV3 cells was assessed using multiplex reverse transcription-PCR. A significant increase in COX-2 mRNA was detected in the cells treated with docetaxel followed by NS398 for 72 h (Fig. 4). No other change in COX-2 was seen with

Table 1 Oligoneucleotide primers used for real-time reverse transcription-PCR analysis

Locus	Sense (5′–3′)	Antisense (5′–3′)	bp
β-Actin	AAGCAGGAGTATGACGAGTCCG	GCCTTCATACATCTCAAGTTGG	559
COX-2	TTCAAATGAGATTGTGGGAAAAT	AGATCATCTCTGCCTGAGTATCTT	304
Bcl-2	TGTGGTATGAAGCCAGACCTCC	CAGGATAGCAGCACAGGATTGG	153
Bax	TTCTGACGGCAACTTCAACTGG	AGGAAGTCCAATGTCCAGCC	135

COX-2, cyclooxygenase-2.

Fig. 1



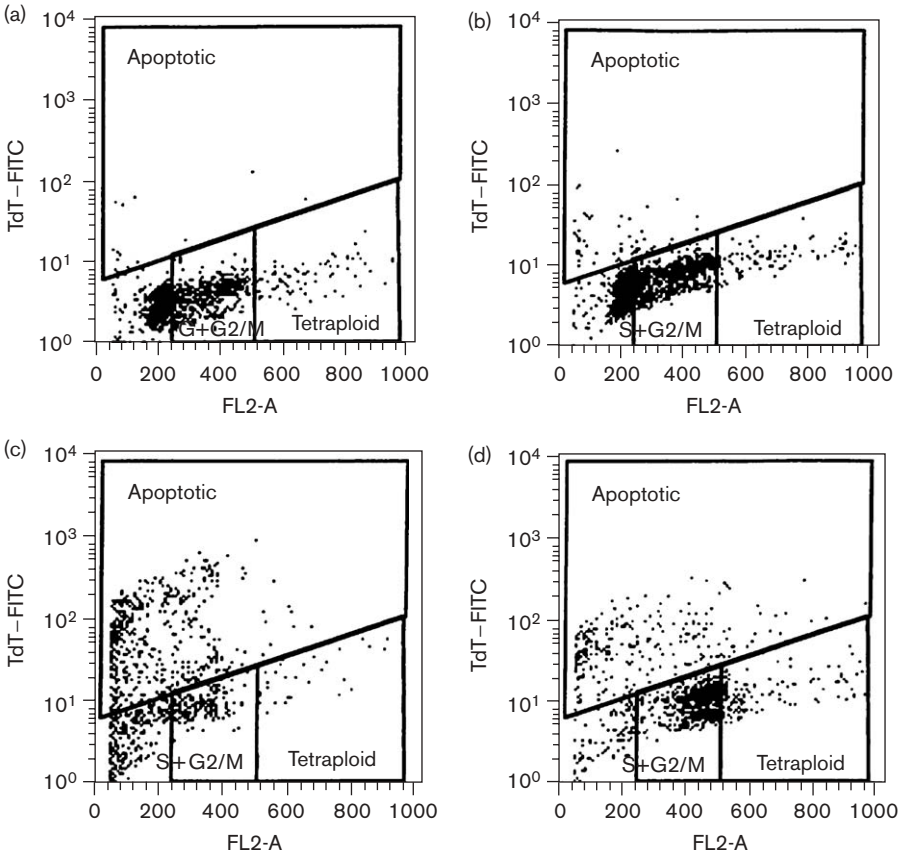
Treatment of ovarian cancer cells with a COX-2 inhibitor before or concurrent with docetaxel reduced the apoptotis induced by the taxane. The ovarian cancer cells MDAH-2774, SKOV3, OVCAR and CaOV-3 were treated with docetaxel and/or NS398 at different doses and schedule. Apoptosis (a and c) and proliferation (b and d) were measured by TUNEL assay and flow cytometry. The following treatments are shown: (A) control, (B) NS398 10 μmol/l for 4 h, (C) docetaxel 10 μmol/l for 24 h, (D) concurrent treatment with NS398 10 μmol/l and docetaxel 10 μmol/l for 24 h, and (E) sequential treatment with NS398 10 μmol/l for 4 h followed by docetaxel 10 μmol/l for 24 h.

the other treatment schedules. The expression of bcl-2 was not significantly altered by any of the treatment modalities. On the other hand, bax mRNA level was significantly reduced in response to the treatment schedule that consisted of concurrently exposing the cells to NS398 and docetaxel (Fig. 4).

Discussion

The cytotoxic effects of COX-2 inhibitors have prompted different investigators to evaluate the effect of combining these drugs with various chemotherapeutic agents. A number of nonsteroidal antiinflammatory drugs (e.g. indomethacin, sulindac, tolmetin, acemetacin, zomeperac and mefenamic acid) have been shown to significantly increase the cytotoxicity of anthracyclines as well as tenoposide, VP-16 and vincristine in human lung and leukemia cell lines [18]. Furthermore, a recent study, evaluating the growth inhibitory effects of sulindac sulfide on human lung cancer cell lines, demonstrated that combining this COX-2 inhibitor with either paclitaxel or cisplatin had a synergistic cytotoxic effect [19]. Similarly, it has been reported that the specific COX-2 inhibitor, nimesulide, when used in combination at clinically achievable concentrations, significantly reduced the IC_{50} values of various anticancer agents in human nonsmall cell lung cancer [20]. This effect was also tested in the clinical setting. In a clinical phase II trial, patients with stage IB to IIIA nonsmall lung cancer were treated with two preoperative cycles of paclitaxel and carboplatin, as well as daily celecoxib, followed by surgical resection [22]. The authors reported that the addition of the selective COX-2 inhibitor might enhance the response to preoperative chemotherapy in that setting. Other investigators have tested the effect of combining docetaxel, zoledronic acid and the COX-2 inhibitor SC236 on human breast cancer cells. They found that the combination of docetaxel with either zoledronic acid or SC236 enhanced the growth inhibition produced by each drug separately. The growth inhibition seen with the triple combination was slightly higher than that seen with either double

Fig. 2

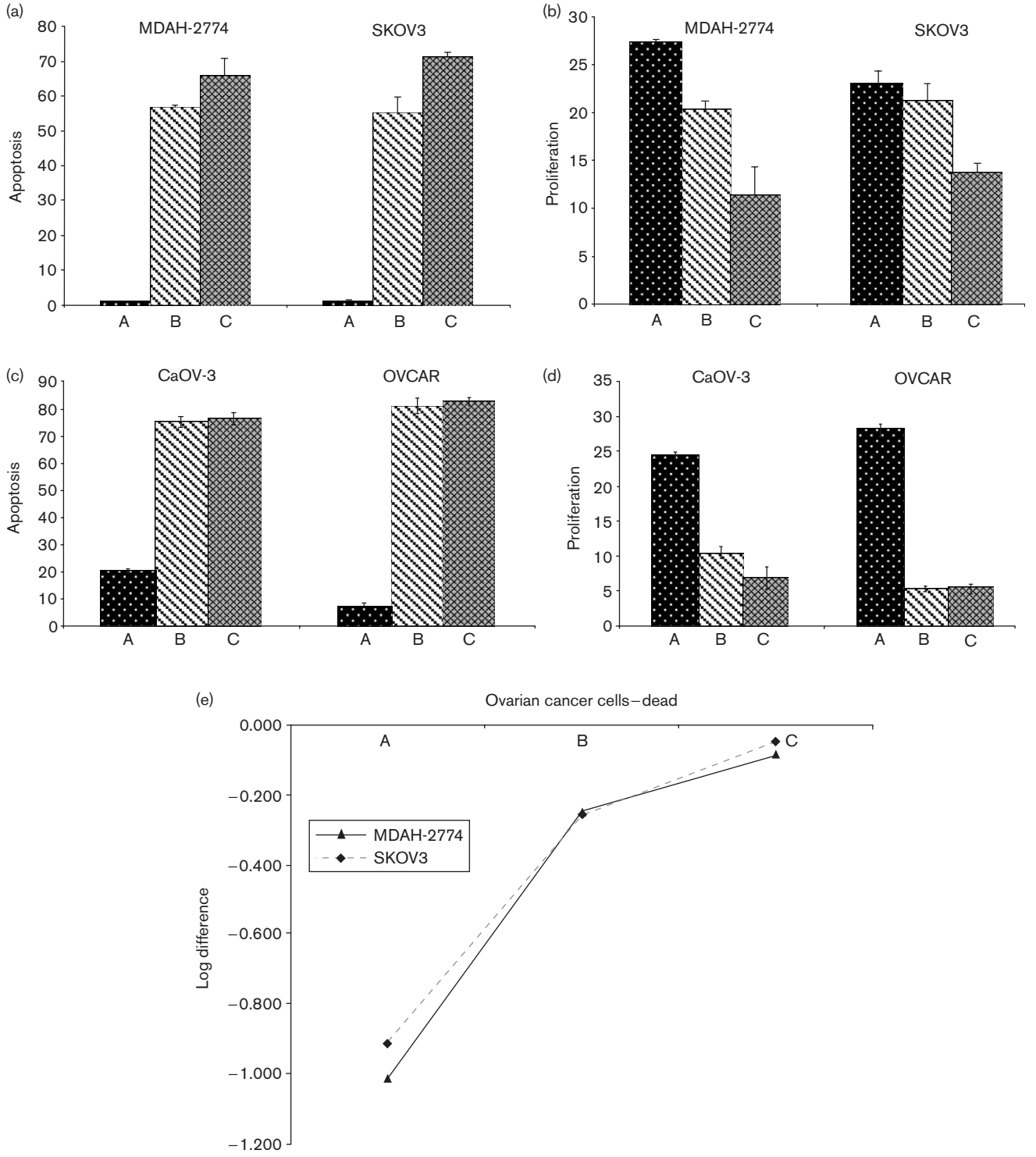


Cell cycle analysis of SKOV3 cells by flow cytometry after various treatments. (a) Control, (b) treatment with NS398 10 μmol/l for 4 h, (c) treatment with docetaxel 10 μmol/l for 24 h, and (d) sequential treatment with NS398 of 10 μmol/l for 4 h then docetaxel of 10 μmol/l for 24 h.

combination [15]. In a previous report, we have established that combining NS398 or aspirin with paclitaxel resulted in a reduction of paclitaxel-induced apoptosis in EOC cell lines. The current study provides evidence that the inhibitory effect is not specific to paclitaxel but is also seen with docetaxel. Interestingly, the described effect is sequence specific. When the cancer cells are exposed to the COX-2 inhibitor before or concurrent with taxane treatment, we observed a reduction in apoptosis. On the other hand, when the cancer cells are exposed to the taxane before exposure to the COX-2 inhibitor, there is no inhibition in the taxane-induced apoptosis; on the contrary, there is an additive cytotoxic effect for some of the cancer cell lines investigated. When looking at the data on proliferation, one needs to be careful not to interpret those as reflecting increased proliferation of the cancer cells in response to treatment with both drugs. It is possible that such treatment resulted in an accumulation of the cells in G_2 phase cycle which was reflected in an increase in the proliferative fraction. Further studies will be done to determine whether the arrested cells will eventually die and thus this combined treatment has switched apoptosis toward another type of cell death.

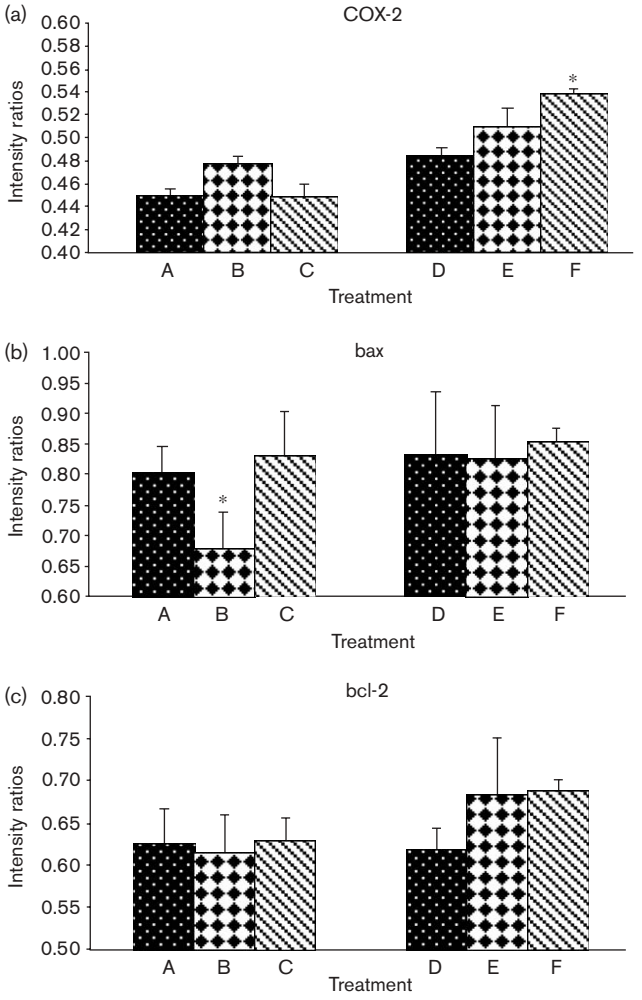
Previous studies have shown that there is some association between the expression of COX-2 and bcl-2 proteins. In rat intestinal epithelial cells, forced COX-2 expression resulted in bcl-2 overexpression and reduced apoptosis [26]. Treatment of EOC cells with prostaglandin E_2 resulted in an increased expression of bcl-2 and reduced apoptosis [27]. It has been speculated that tumor cells that overexpress COX-2 tend to be more resistant to apoptosis because of increased bcl-2 levels. Studies have suggested that taxanes induce apoptotic cell death through a bcl-2-associated mechanism. In various cancer cell lines, taxane treatment induced bcl-2 hyperphosphorylation and apoptosis, and reduced bcl-2/bax dimerization. In our experimental model, we hypothesized that inhibiting COX-2 using NS398 might potentiate the apoptotic effect of taxanes by further reducing bcl-2 levels [28]. The data presented, however, reveal no change in bcl-2 expression. On the other hand, pretreating the cells with a COX-2 inhibitor before docetaxel

Fig. 3



Treatment of ovarian cancer cells with a COX-2 inhibitor after docetaxel increased the taxane-induced apoptosis. The ovarian cancer cells MDAH-2774, SKOV3, OVCAR and CaOV-3 were treated with docetaxel and/or NS398 at different doses and schedule. Apoptosis (a and c) and proliferation (b and d) were measured by TUNEL assay and flow cytometry. Cell viability was assessed using Trypan blue and the log difference of dead cells after treatment was computed and plotted (e). The following treatments are shown: (A) control, (B) docetaxel of 0.1 μmol/l for 24 h then cells harvested at 96 h, and (C) doctaxel of 0.1 μmol/l for 24 h followed by NS398 of 100 μmol/l for 72 h (cells harvested at 96 h).

Fig. 4



Changes in bax, bcl-2 and COX-2 mRNA levels in response to treatment with docetaxel and NS398. SKOV3 cells were treated with NS398 and/or docetaxel. COX-2, bax and bcl-2 mRNA levels were measured using mutiplex reverse transcription-PCR and intensity ratios with respect to β-actin cDNA were plotted. The treatments presented on these graph are as follows: (A) control with harvesting at 24 h, (B) concurrent treatment with 10 μmol/l of NS398 and 10 μmol/l of docetaxel for 24 h, (C) docetaxel 10 μmol/l for 24 h, (D) control with harvesting at 96 h, (E) docetaxel 0.1 μmol/l for 24 h then cells harvested at 96 h, and (F) docetaxel 0.1 μmol/l for 24 h followed by NS398 100 μmol/l for 72 h (cells harvested at 96 h). (∗ indicates $P < 0.05$).

resulted in a reduction of bax mRNA levels. One might wonder whether the decreased expression of this proapoptotic gene is the mechanism underlying the inhibition of the taxane-induced apoptosis by COX-2 inhibitor. Future experiments will be evaluating protein levels as well as protein phosphorylation in an attempt to explain the phenomena observed in the current experiments. In addition, other apoptotic pathways need to be investigated.

Previous studies have shown that microtubule-acting agents impact COX-2 expression in cells [16]. In fact, treatment with taxanes was shown to induce COX-2 expression by both stimulating transcription and stabilizing mRNA [29]. A number of studies have shown that COX-2 overexpression in cancer cells can promote their proliferation, reduce their apoptotic death and enhance their metastatic potential. Therefore, it is possible that the induction of COX-2 expression by taxane paradoxically benefit the malignant cells and reduce the efficacy of these chemotherapy agents [30]. Accordingly, the administration of a COX-2 inhibitor with a taxane might reverse this effect and improve the cytotoxic effect of the chemotherapy. This was demonstrated in an experimental lung cancer model in which the combined use of a selective COX-2 inhibitor along with conventional chemotherapy resulted in more tumor growth inhibition than with chemotherapy alone [31]. The effect of taxane therapy on COX-2 expression in the lung cancer cells was not evaluated in that report. Similar to what has been reported in the lung cancer model, this study demonstrates that the sequential treatment of ovarian cancer cells with docetaxel followed by NS398 resulted in an increase in apoptotic cell death. Docetaxel treatment alone, however, had no significant impact on COX-2 expression in any of the EOC cell lines studied.

In addition to inducing apoptosis, part of the growth inhibitory effect of the traditional nonsteroidal anti-inflammatory drugs on colorectal carcinoma cells is due to their effect on the cell cycle [32]. These drugs induce G_0/G_1 arrest by regulating some of the proteins that control the progression through the cell cycle [33]. Similarly, a recent study noted that NS398 inhibited the growth of ovarian carcinoma cells by inducing a G_0/G_1 arrest independent of COX2 expression [34]. It is well established that one of the mechanisms of action of taxane is the induction of a G_2 arrest. We sought to determine whether, in our experimental model, the pretreatment of EOC cells with NS398 resulted in a significant G_0/G_1 arrest that protected the cells from reaching the G_2 phase where the taxane will exert most of their cell kill. Flow cytometry analysis revealed that this was not the case. The treatment of EOC cells with docetaxel and NS398 caused apoptosis and sequestering in G_2 phase, but no significant G_0/G_1 arrest.

In conclusion, the over expression of COX-2 in epithelial ovarian carcinoma and its known effect on cell growth and proliferation makes it a good potential therapeutic target. The reduction in taxane-induced apoptosis seen when the ovarian cancer cells were pretreated with a COX-2 inhibitor, however, is a reason for concern. Future studies need to determine whether the concurrent or sequential treatment of docetaxel and NS398 are truly inhibiting cell death or delaying it by switching the cells toward another death mechanism. Interestingly, this effect seems to be sequence dependent. In fact, cellular apoptosis was increased when the taxane is used before

the COX-2 inhibitor. This increase in cell death can provide the scientific basis for clinical trials of drug combinations in ovarian cancer that include taxanes and COX-2 inhibitors. Although such combinations may be beneficial from a cancer therapy perspective, they need to be weighed against the risk of serious cardiovascular side effects recently attributed to the COX-2 inhibitors.

References

1 McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, *et al*. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with State III and Stage IV ovarian cancer. *N Engl J Med* 1996; **334**:1–6.

2 Diaz JF, Andreu JM. Assembly of purified GDP-tubulin into microtubules induced by taxol and taxotere: reversibility, ligand stoichiometry, and competition. *Biochemistry* 1993; **32**:2747–2755.

3 Moos PJ, Fitzpatrick FA. Taxanes propagate apoptosis via two cell populations with distinctive cytological and molecular traits. *Cell Growth Differ* 1998;**9**:687–697.

4 Vasey PA, Jayson GC, Gordon A, Gabra H, Coleman R, Atkinson R, *et al*. Phase III randomized trial of doctaxel–carboplatin versus paclitaxel–carboplatin as first-line chemotherapy for ovarian carcinoma. *J Natl Cancer Inst* 2004;**96**:1682–1691.

5 DuBois RN, Radhika A, Reddy B, Entingh AJ. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. *Gastroenterology* 1996; **110**:1259–1262.

6 Kujubu DA, Fletcher BS, Varnum BS, Lim RW, Herschman HR. TS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthasel/cyclooxygenase homologue. *J Biol Chem* 1991; **266**:12866–12872.

7 Chen G, Wilson R, McKillop JH, Walker JJ. The role of cytokines in the production of prostacyclin and thromboxane in human monomuclear cells. *Immunol Invest* 1994; **223**:269–279.

8 Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, *et al*. Overexpression of COX-2 is sufficient to induce tumorgenesis in transgenic mice. *J Biol Chem* 2001; **276**:18563–18569.

9 Greenberg ER, Baron JA, Freeman DH Jr, Mandel JS, Haile R. Reduced risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study Group. *J Natl Cancer Inst* 1993; **85**:912–916.

10 Thun MJ, Manboodiri MM, Callee EE, Flanders WD, Heath CW Jr. Aspirin use and risk of fatal cancer. *Cancer Res* 1993; **53**:1322–1327.

11 Rosenberg L, Palmer JR, Rao RS, Coogan PF, Strom BL, Zauber AG, *et al*. A case-control study of analgesic use and ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2000; **9**:933–937.

12 Maolic S, Liagre B, LeBail JC, Beneytout JL. Dose dependent modulation of apoptosis and COX-2 expression in human 1547 osteosarcoma cells by NS-398, a selective COX inhibitor. *Int J Oncol* 2001; **18**:533–540.

13 Elder DJ, Halton DE, Crew TE, Paraskeva C. Apoptosis induction and COX-2 selective non-steroidal anti-inflammatory drug NS-398. *Int J Cancer* 2000; **86**:553–560.

14 Rodriguez-Burford C, Barnes MN, Oelschlager DK, Meyers RB, Talley LI, Partridge EE, *et al*. Effects of nonsteroidal anti-inflammatory agents (NSAIDs) on ovarian carcinoma cell lines: preclinical evaluation of NSAIDs as chemopreventatie agents. *Clin Cancer Res* 2002; **8**:202–209.

15 Witters LM, Crispino J, Fraterrigo T, Green J, Lipton A. Effect of the combination of docetaxel, zoledronic acid, and a COX-2 inhibitor on the growth of human breast cancer cell lines. *Am J Clin Oncol* 2003; **26**:S92–S97.

16 Olsen SR. Taxanes and COX-2 inhibitors: from molecular pathways to clinical practice. *Biomed Pharmacother* 2005; **59** (**Suppl 2**):S306–S310.

17 Xu XC. COX-2 Inhibitors in cancer treatment and prevention, a recent development. *Anticancer Drugs* 2002; **13**:127–137.

18 Duffy CP, Elliot CJ, O'Connor RA, Heenan MM, Coyle S, Cleary IM, *et al*. Enhancement of chemotherapeutic drug toxicity to human tumor cells *in vitro* by a subset of non-steroidal anti-inflammatory drugs (NSAIDs). *Eur J Cancer* 1998; **34**:1250–1259.

19 Soriano AF, Helfrich B, Chan DC, Heasley LE, Bunn PA, Chou TC. Synergistic effects of new chemopreventative agents and conventional cytotoxic agents against human lung cancer cell lines. *Cancer Res* 1999; **59**:6178–6184.

20 Hida T, Kozaki K, Muramatsu H, Masuda A, Shimizu S, Mitsudomi T, *et al*. Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytoxicity of various anticancer agents in non-small cell lung cancer cell lines. *Clin Cancer Res* 2000; **6**:2006–2011.

21 Munkarah AR, Genhai Z, Morris R, Baker VV, Deppe G, Diamond MP, *et al*. Inhibition of paclitaxel-induced apoptosis by the specific COX-2 inhibitor, NS398, in epithelial ovarian cancer cells. *Gynecol Onc* 2003; **88**:429–433.

22 Altorki NK, Keresztes RS, Port JL, Libby DM, Korst RJ, Flieder DB, *et al*. Celecoxib, a selective cyclo-oxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer. *J Clin Oncol* 2003; **21**:2645–2650.

23 Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 1992; **119**:493–501.

24 Ben-Sasson SA, Sherman Y, Gavrieli Y. Identification of dying cells: *in situ* staining. *Methods Cell Biol* 1995; **46**:29–39.

25 Li X, Traganos F, Melamed MR, Darzynkiewicz Z. Single-step procedure for labeling DNA strand breaks with fluorescein- or BODIPY-conjugated deoxynucleotides: detection of apoptosis and bromodeoxyuridine incorporation. *Cytometry* 1995; **20**:172–180.

26 Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandins endoperoxide synthase 2. *Cell* 1995; **83**:493–501.

27 Munkarah AR, Morris R, Baumann P, Deppe G, Malone J, Diamond MP, *et al*. Effects of prostaglandin E_2 on proliferation and apoptosis of epithelial ovarian cancer cells. *J Soc Gynecol Invest* 2002; **9**:168–173.

28 Ganansia-Leymarie V, Bischoff P, Bergerat JP, Holl V. Signal transduction pathways of taxanes-induced apoptosis. *Curr Med Chem Anticancer Agents* 2003; **3**:291–306.

29 Subbaramaiah K, Hart JC, Norton L, Dannenberg AJ. Microtubule interfering agents stimulate the transcription of cyclooxygenase-2. Evidence for involvement of ERK1/2 and mitogen-activated protein kinase pathways. *J Biol Chem* 2000; **275**:1438–1445.

30 Subbaramaiah K, Marmo TP, Dixon DA, Dannenberg AJ. Regulation of cyclooxgenase-2 mRNA stability by taxanes: evidence for involvement of p38, MAPKAPK-2, and HuR. *J Biol Chem* 2003; **278**:37637–37647.

31 Hida T, Kozaki K, Ito H, Miyaishi O, Tatematsu Y, Suzuki T, *et al*. Significant growth inhibition of human lung cancer cells both *in vitro* and *in vivo* by the combined use of a selective cyclooxygenase 2 inhibitor, JTE-522, and conventional anticancer agents. *Clin Cancer Res* 2002; **8**:2443–2447.

32 Elder DJ, Paraskeva C. Induced apoptosis in the prevention of colorectal cancer by non-steroidal anti-inflammatory drugs. *Apoptosis* 1999; **4**: 365–372.

33 Goldberg Y, Nassif II, Pittas A, Tsai LL, Dynlacht BD, Rigas B, *et al*. The anti-proliferative effect of sulindac and sulindac sulfide on HT-29 colon cancer cells: alterations in tumor suppressor and cell cycle-regulatory proteins. *Oncogene* 1996; **12**:893–901.

34 Denkert C, Furstenberg A, Daniel PT, Koch I, Kobel M, Weichert W, *et al*. Induction of G_0/G_1 cell cycle arrest in ovarian carcinoma cells by the anti-inflammatory drug NS-398, but not by COX-2-specific RNA interference. *Oncogene* 2003; **22**:8653–8661.