**BASIC SCIENCE: GYNECOLOGY**

**Nonsteroidal antiinflammatory drugs and progestins synergistically enhance cell death in ovarian epithelial cells**

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**OBJECTIVE:** There is growing evidence that progestins and nonsteroidal antiinflammatory drugs (NSAIDs) may prevent ovarian cancer. Because both induce apoptosis, we investigated the potential for synergistic impact of combined drug treatment on cell death.

**STUDY DESIGN:** Using normal and malignant human ovarian epithelial cells and an NSAID-sensitive human colon cancer cell line, we evaluated the effects of progestins and NSAIDs alone and in combination on apoptosis.

**RESULTS:** Both progestins and NSAIDs dose dependently inhibited cell growth ($P < .0001$). Doses of NSAIDs or progestins that independently reduced cell viability by less than 30% synergistically reduced cell viability by 70–95% when combined. Similarly, the NSAID/progestin combination conferred 4- to 18-fold ($P < .05$) increased apoptosis over either treatment alone.

**CONCLUSION:** Our results suggest it may be possible to combine progestins and NSAIDs to achieve ovarian cancer prevention at lower doses of each than are required for single administration, thereby lessening the risk of side effects posed by these agents.

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There is significant potential to decrease ovarian cancer incidence and mortality through chemoprevention. Epidemiological evidence has shown that routine use of the combination estrogen-progestin oral contraceptive pill (OCP) confers a 30-50% reduction in the risk of developing epithelial ovarian cancer, suggesting that an effective pharmacological approach for the chemoprevention of ovarian cancer is possible. Investigations by our group have elucidated a mechanism that may be responsible for the ovarian cancer preventive effects of the OCP. Specifically, we have discovered that the progestin component of the OCP may be functioning as a chemopreventive agent in the ovarian surface epithelium by triggering apoptosis, a biological effect that is well known to be associated with cancer prevention. This raises the possibility that progestins enhance the clearance of genetically damaged ovarian epithelial cells, thereby significantly lowering ovarian cancer risk.

Several lines of human evidence support an ovarian cancer preventive role for progestins, including the following: (1) evidence of a 60% reduction in the risk of nonmucinous ovarian cancer in women who have used depomedroxyprogesterone acetate, a progestin-only contraceptive; (2) evidence that twin gestation, which is associated with higher circulating levels of progesterone, is more protective against subsequent ovarian cancer risk than singleton pregnancy; and (3) evidence that use of progestin-potent OCPs confers twice the ovarian cancer protective effect as use of OCPs containing weak progestins. Taken together, these data provide a strong rationale to evaluate progestins as ovarian cancer preventives and suggest that a pharmacological regimen that has enhanced chemopreventive biological potency in the ovarian epithelium will be more effective than lower-potency regimens.

The finding that progestins activate apoptosis in the ovarian epithelium suggests that it may be possible to develop other agents with similar biological effects as ovarian cancer preventives. In this regard, there is a growing body of epidemiological and laboratory evidence in support of nonsteroidal antiinflammatory drugs (NSAIDs) and other antiinflammatory agents as cancer-preventive agents for a variety of cancers, including ovarian cancer. Case-control comparisons suggest a reduction in ovarian cancer risk associated with use of NSAIDs as well as acetaminophen.

The molecular basis for the protective effect of these agents has not been well defined but may involve a direct chemopreventive biological effect on the ovarian epithelium similar to that induced by progestins. It has been shown, for exam-
ple, that NSAIDs induce apoptosis in cells derived from human ovarian epithelium, similar to progestins.18-20 Based on these data, we have hypothesized that progestins and NSAIDs target the early steps of carcinogenesis in the ovarian epithelium by activating apoptosis, thereby clearing dysplastic cells and resulting in effective cancer prevention. In this study, we sought to determine whether the effects of progestins and NSAIDs would be synergistic when these agents are combined. We show that in ovarian epithelial cells these 2 classes of drugs have a synergistic effect on cell death.

**Materials and Methods**

All culture media, sera, and reagents were purchased from Invitrogen (Carlsbad, CA), except MCDB105 and insulin, which were purchased from Sigma (St Louis, MO). All NSAIDs, acetaminophen, and progestins were purchased from Sigma, except celecoxib, which was purchased from LKT Laboratories (St Paul, MN).

**Cell culture**

The ovarian cancer cell lines OVCAR-3 (purchased from the American Type Culture Collection, Manassas, VA) and OVCAR-5 (kindly provided by Dr Tom Hamilton, Fox Chase Cancer Center, Philadelphia, PA) were grown in RPMI 1640 medium with glutamine supplemented with 10% heat-inactivated fetal bovine serum (FBS), 10 µg/mL insulin,
and penicillin/streptomycin. HIO-118V, a transformed human ovarian surface epithelial cell line transfected with Simian virus 40 antigen to extend its life span\(^1\) (kindly provided by Dr Vimla Band, University of Nebraska Medical Center [Omaha, NE], with permission of Dr Andrew Godwin, Fox Chase Cancer Center) and normal ovarian epithelial cell cultures (NOE cells; harvested from a normal human ovary during diagnostic surgery under the approval of the institutional review board) were grown in a 1:1 mixture of Medium 199 and MCDB105 with 15% heat-inactivated FBS, glutamine, 10 ng/mL epidermal growth factor, and penicillin/streptomycin. The HT-29 colon cancer cell line (purchased from American Type Culture Collection) was grown in McCoy’s 5A medium with 10% heat-inactivated FBS and penicillin/streptomycin. All cell lines were maintained in 5% CO\(_2\) at 37°C.

**Cell viability/proliferation testing**

Cells were seeded overnight in 96-well plates at 2500-5000 cells/well. The culture medium was replaced with fresh medium containing experimental agents (progestins and/or NSAIDs in varying concentrations to yield 20-30% decreased proliferation in each respective cell line). Cells were treated for 72 hours, at which time untreated controls were nearly confluent. All treatments were performed in quadruplicate. Viability was tested using the formazan dye-based 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphonyl)-2H-tetrazolium, inner salt (MTS) assay (Promega, Madison, WI) according to the manufacturer’s specifications. The plates were incubated at 37°C until the untreated wells exhibited an A\(_{490}\) of 0.7-0.9. Wells containing medium alone were used as blanks. Viability was expressed as a percentage of untreated controls. All experiments were repeated at least 3 times.

**Detection of apoptosis by activated caspase-3 and TUNEL assays**

OVCAR-3, OVCAR-5, and HIO-118V cells were seeded overnight into 60 mm dishes at 2.8 \(\times\) 10\(^5\), 1.6 \(\times\) 10\(^5\) and 1.6 \(\times\) 10\(^5\) cells/dish, respectively. The culture medium was then replaced with fresh medium containing progestrone, sulindac sulfide, or celecoxib or progestrone combined with sulindac sulfide or celecoxib and incubated for 48 hours. Vehicle concentrations did not exceed 0.125% for dimethylsulfoxide and 0.32% for ethanol. All cells, floating and adherent, were collected, washed, fixed, and stained using either fluorescein isothiocyanate (FITC)-labeled antibody to active caspase-3 (APO Active 3) purchased from Cell Technology (Beverly, MA) or deoxynucleotidyltransferase deoxyuridine 5-triphosphate (dUTP) nick end labeling (TUNEL) using the APO Direct kit purchased from BD Pharmingen (San Diego, CA). Both assays were performed according to the manufacturers’ protocols, with the following modification to the caspase-3 assay: 0.1% saponin was used in the rinse buffer. Ten thousand cells were measured by FACS Calibur (BD Biosciences, Franklin Lakes, NJ), and the data were analyzed using Cellquest 3.3 software (BD Biosciences).

FITC fluorescence was collected through a 488 bandpass filter and forward and side scatter were analyzed on a linear scale. For the caspase-3 assay, histograms, on a logarithmic scale, were used to assess the percentage of caspase-3–labeled cells for each treatment group. Gates were based on the untreated controls. For the TUNEL assay, each sample was gated to include only a singlet population using dual parameters of deoxyribonucleic acid (DNA) width (x-axis) and DNA area (y-axis). In assessing apoptosis, the resultant gates were used to generate dual parametric graphs of DNA area (x-axis) and FITC-dUTP (y-axis). Gates were based on increased dUTP-FITC labeling compared with the untreated controls. In both experiments, results are expressed as fold increase over controls. Both sets of experiments were repeated at least 3 times and/or run in triplicate.

**Determination of synergy**

Data were analyzed isobolographically using CalcuSyn software (Biosoft, Cambridge, UK) to characterize the inhibitory effect drug combinations had on the cell lines. Raw data for each drug or drug combination were entered singly to generate a median effect plot. From this plot, the combination index (CI) equation was applied to determine whether the drug effects were additive, synergistic, or antagonistic. CI values of less than 1, 1, or greater than 1 indicated synergy, additivity, or antagonism, respectively.

**Fluorescent caspase assay**

Fluorescent caspase-3 and -9 substrates and their inhibitors were obtained from Calbiochem (San Diego, CA). Cells were treated for 20-24 hours at drug concentrations that killed most cells as measured by MTS assay at 72 hours. Cell lysates were harvested and combined with a reaction buffer and the uncleaved, fluorescent substrate, according to the man-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cell lines</th>
<th>OVCAR-3</th>
<th>OVCAR-5</th>
<th>HT-29</th>
<th>NOE</th>
<th>HIO-118V</th>
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<tbody>
<tr>
<td>Progestin</td>
<td></td>
<td></td>
<td></td>
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<td>Levonorgestrel</td>
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<td>197</td>
<td>150</td>
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<td>Norethindrone</td>
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<td>464</td>
<td>174</td>
<td>141</td>
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<tr>
<td>Progesterone</td>
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<td>34</td>
<td>56</td>
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<td>87</td>
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<tr>
<td>NSAIAND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
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<td>37</td>
<td>60</td>
<td>31</td>
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<tr>
<td>Sulindac sulfide</td>
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<td>80</td>
<td>77</td>
<td>110</td>
<td>190</td>
<td>169</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td></td>
<td>4100</td>
<td>2700</td>
<td>5600</td>
<td>4400</td>
<td>3100</td>
</tr>
</tbody>
</table>

RESULTS
Treatment with progestins and NSAIDs inhibits cell growth

The 2 ovarian cancer cell lines, OVCAR-3 and OVCAR-5, a primary normal ovarian epithelial cell culture, NOE, a transformed ovarian epithelial cell line, HIO-118V, and the colon cancer cell line, HT-29, were tested in dose-response experiments for the effects of progestins and NSAIDs.

All cell types were growth inhibited in a dose-dependent manner when exposed to progestins \((P < .0001; \text{Figure 1, A})\). Relative to the normal and transformed ovarian epithelial cell cultures, the cancer cell lines OVCAR-3, OVCAR-5, and HT-29 were less sensitive to the progestins levonorgestrel and norethindrone and more sensitive to progesterone. The 50% lethal dose (LD\(_{50}\)) values from levonorgestrel treated cells ranged from 70 \(\mu\)M in the HIO-118V cells to 359 \(\mu\)M in the HT-29 cells, whereas norethindrone LD\(_{50}\) values ranged from 141 \(\mu\)M in the OVCAR-5 and HT-29 cells (Table 1).

In response to progesterone treatments, LD\(_{50}\) values ranged from 34 \(\mu\)M in the most sensitive OVCAR-5 cells to 121 \(\mu\)M for the least responsive NOE cells (Table 1). The effect of the NSAIDs sulindac sulfide, celecoxib, and acetaminophen on cell viability is shown in Figure 1, B. Again, all cell types were growth inhibited in a dose-dependent manner \((P < .0001; \text{Figure 1, B})\).

In contrast to the finding with progestins, the cancer cell lines were more sensitive to NSAIDs than were the nonmalignant ovarian epithelial cell cultures. In particular, relatively low doses of celecoxib treatment produced the most cytotoxic effects, with HT-29 and OVCAR-3 cells showing the most sensitivity (LD\(_{50}\) = 31 \(\mu\)M and 37 \(\mu\)M, respectively; Table 1).

Combination treatment with NSAIDs and progesterone has synergistic effect on cell viability

Doses of NSAIDs and progestins that cause minimal loss of cell viability (<30% decrease) in the MTS assays were shown to cause dramatic loss of cell viability when combined (<75% viability).
compared with control; Figures 2 and 3). Combining the drug vehicles had no effect on cell viability. Isobolographic analysis of the results using CalcuSyn software (Biosoft) generated combination index values less than 1 (0.3–0.8), demonstrating that the effect of combining progesterone and an NSAID is synergistic.

**NSAIDs and progesterone induce caspase-3 and cause morphological changes consistent with apoptosis**

Using the dosages of progesterone and NSAIDs described in Figures 2 and 3, treated cells were examined microscopically for morphological signs of apoptosis including chromatin condensation, anoikis, and blebbing. By 48 hours, following initiation of treatment, all cell types that were treated with an NSAID/progestin combination showed morphological signs of apoptosis (Figure 4, A and B, fourth panel).

Marked induction of caspase-3 was observed using flow cytometry following combination treatments of either progesterone with celecoxib or progesterone with sulindac sulfide (Table 2 and Figure 4, C and D, fourth panel). OVCAR-5 and HIO-118V cells treated with the progesterone/celecoxib combination demonstrated the most significant increases in activated caspase-3 values (14.3- and 17.9-fold over control levels, \( P < .01 \)), whereas OVCAR-3 showed a 3.9-fold increase over its control (\( P < .01 \); Table 2).

In cells treated with the progesterone/sulindac sulfide combination, similar increases in caspase-3 were demonstrated in OVCAR-5 and OVCAR-3 cells (9.2 and 5.2-fold, respectively, \( P < .01 \)). In the HIO-118V cells, however, only a 2-fold increase was achieved relative to progesterone alone. Thus, the levels of activated caspase-3 were significantly higher in most cells treated with a progesterone/NSAID combination compared with the single agent treatments.

The exception to this trend was observed in HIO-118V cells treated with progesterone/sulindac sulfide; this cell line had low caspase-3 values, even with the combination treatment. The activation of caspase-3 was analyzed for synergy in the combination treatments, and all 3 cell lines demonstrated synergism, with CI values less than 0.6.

Finally, lysates from OVCAR-3 and OVCAR-5 cells treated with either progesterone or sulindac sulfide were evaluated for caspase-3 and -9 activities using a kinetic assay and fluorescent substrates (data not shown). This assay showed increases in

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**FIGURE 3**

**Progestosterone-celecoxib synergy**

Viability for 5 cell lines is shown after 72 hours of treatment with progesterone (P4) and celecoxib (Cel), singly or in combination, using MTS reagent to determine the number of viable cells relative to untreated cells (UT) (100%) and empty wells (0%). Doses for each cell line were determined based on proliferation reduction of 20–30%. A, Ovarian cancer cell lines (OVCAR-3 and OVCAR-5). B, Normal ovarian cell lines (NOE and HIO-118V). C, Colon cancer line (HT-29). Isobolographic analysis (CalcuSyn software; Biosoft, Cambridge, UK) demonstrated synergy for all drug combinations (combination index values <0.8). Bars represent mean ± SE. The asterisk indicates \( P < .05 \) compared with untreated cells, by analysis of variance.

caspase-3 activity consistent with the flow cytometry experiments (2.4- to 6-fold) and a small but reproducible increase in caspase-9 activity (1.5-fold). Caspase-9 is a low abundance enzyme whose activity is therefore difficult to measure. The presence of activated caspase-9 is suggestive of apoptosis occurring through the mitochondria (the intrinsic cell death pathway).

**NSAIDs and progesterone induce DNA strand breakage as revealed by TUNEL**

The TUNEL assay further confirmed that cell death caused by the combination of an NSAID and progesterone was primarily due to apoptosis (Table 2). OVCAR-5 cells treated with a combination of sulindac sulfide and progesterone exhibited a nearly 16-fold increase in TUNEL-positive cells relative to untreated cells ($P < .01$), whereas the...
TABLE 2

<table>
<thead>
<tr>
<th>Treatments</th>
<th>OVCAR-3</th>
<th>OVCAR-5</th>
<th>HIO-118V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated caspase-3 induction, relative fold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.3 ± 0.17</td>
<td>2.3 ± 0.12 (^a)</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>2.5 ± 0.24(^b)</td>
<td>3.0 ± 0.18(^b)</td>
<td>6.2 ± 1.07(^b)</td>
</tr>
<tr>
<td>Sulindac sulfide</td>
<td>1.4 ± 0.10</td>
<td>1.1 ± 0.08</td>
<td>1.7 ± 1.07</td>
</tr>
<tr>
<td>Progesterone plus celecoxib</td>
<td>3.9 ± 0.28(^b)</td>
<td>14.3 ± 1.34(^a)</td>
<td>17.9 ± 0.71(^b)</td>
</tr>
<tr>
<td>Progesterone plus sulindac sulfide</td>
<td>5.2 ± 0.19(^b)</td>
<td>9.2 ± 0.21(^b)</td>
<td>2.0 ± 0.11</td>
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<tr>
<td>TUNEL induction, relative fold</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.1 ± 0.15</td>
<td>4.1 ± 1.15</td>
<td>1.4 ± 1.00</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>1.0 ± 0.10</td>
<td>7.2 ± 1.03(^a)</td>
<td>2.9 ± 0.33(^a)</td>
</tr>
<tr>
<td>Sulindac sulfide</td>
<td>1.3 ± 0.30</td>
<td>1.2 ± 0.38</td>
<td>1.8 ± 0.20</td>
</tr>
<tr>
<td>Progesterone plus celecoxib</td>
<td>1.2 ± 0.17</td>
<td>42.5 ± 5.97(^b)</td>
<td>8.3 ± 0.96(^b)</td>
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<tr>
<td>Progesterone plus sulindac sulfide</td>
<td>2.8 ± 0.37(^b)</td>
<td>15.8 ± 0.81(^b)</td>
<td>2.3 ± 0.15</td>
</tr>
</tbody>
</table>

In the activated caspase-3 assay, cells were incubated 48 hours and treated with the following, either singly or in combination: Progesterone (Prog), Celecoxib (Cele), HO-118V, 40 μM Prog and 80 μM Cele; OVCAR-3 and OVCAR-5, 30 μM Prog and 40 or 60 μM Cele, respectively; Progesterone (Prog), Sulindac sulfide (SS), HO-118V, 45 μM Prog and 125 μM SS; OVCAR-3 and OVCAR-5, 30 μM Prog and 80 μM SS. Data are presented as mean ± SEM. In the TUNEL assay, OVCAR-3 cells were incubated 28 hours and OVCAR-5 and HIO-118V cells were incubated 32 hours and were treated singly or in combination as follows: Prog-Cele, HO-118V, 40 μM Prog and 80 μM Cele; OVCAR-3 and OVCAR-5, 30 μM Prog and 30 or 60 μM Cele, respectively; Prog-SS, HO-118V, 45 μM Prog and 125 μM SS; OVCAR-3 and OVCAR-5, 30 μM Prog and 80 μM SS. Data are presented as mean ± SEM.

*NSAID, nonsteroidal antiinflammatory drug; OVCAR, ovarian cancer cell lines.

\(^a\) P < .05; \(^b\) P < .01 relative to untreated cells, by analysis of variance.


OVCAR-3 cells and HIO-118V cells showed a 2.8-fold (P < .01) increase and a 2.3-fold nonsignificant change, respectively. The celecoxib/progesterone combination caused a range of responses among the cell lines, ranging from no increase in DNA damage in OVCAR-3 cells to an 8-fold increase in HIO-118V cells to a dramatic 42-fold increase in OVCAR-5 cells (P < .01). The results are generally consistent with the flow cytometry data for caspase-3 activity.

COMMENT

There is an urgent need for a novel approach that arrests or reverses early events in ovarian carcinogenesis. To date, ovarian cancer therapies have been directed almost exclusively toward the latter aspects of ovarian carcinogenesis and have yielded only marginal improvements in long-term survival.\(^2,2\)

Most women with ovarian cancer have an advanced stage at diagnosis, often characterized by a marked tumor burden comprising many cubic centimeters of bulk disease. Considering that each cubic centimeter of tumor contains as many as 1 billion cells, many cell doublings have occurred by the time an ovarian cancer is typically diagnosed, providing an immense opportunity for tumors to harbor cells with genetic heterogeneity and for the emergence of chemotherapy-resistant clones of cells, which may fail to respond to conventional chemotherapy.

This readily explains the clinical course of women with ovarian cancer because many will experience recurrence and will succumb to the disease as chemoresistant clones of ovarian cancer cells emerge. Consequently, the battle against ovarian cancer may be more effectively fought and won via a pharmacological chemopreventive approach that targets the early steps in ovarian carcinogenesis, in which cellular heterogeneity is less likely, and the molecular pathways mediating the effects of chemopreventives are more likely to be intact.

Among the various molecular targets for reversal of early ovarian carcinogenesis, the apoptosis pathway holds great promise. The apoptosis pathway is arguably one of the most important in vivo mechanisms for cancer prevention. Activation of apoptosis leads to efficient disposal of cells that have undergone irreparable genetic damage and that are prone to neoplastic transformation.\(^23\) It is thus a key molecular pathway for elimination of pre-malignant cells in vivo.

Pharmacological agents that selectively enhance apoptosis have been shown to lower the risk of a variety of cancers in animals and humans.\(^24,25\) In addition, in animal models of cancer as well as in humans, the efficacy of cancer preventive agents correlates with the degree of apoptosis.\(^24,25\) Conversely, mutations in genes involved in the apoptosis pathway are associated with enhanced cancer risk.\(^28\) Importantly, with regard to the ovary, the apoptotic pathway is markedly activated in the ovarian surface epithelium by an oral contraceptive, a potent ovarian cancer preventive. This suggests that agents that selectively activate apoptosis in the ovarian surface epithelium may be potent ovarian chemopreventives.\(^4,5\)

The current study demonstrates that progestins and NSAIDs activate apoptosis in cells derived from the ovarian surface epithelium. Our findings are consistent with our prior observation in primates in vivo.\(^4,5\) They are also in agreement with investigations by others that demonstrate induction of apoptosis in ovarian epithelial cells and cancer cell lines in vitro by progestosterone\(^29,31\) and by the antiinflammatory agents aspirin, acetaminophen, and sulindac sulfide.\(^18,19\)

Additionally, our study is the first to demonstrate that the combination of a progestin and NSAIDs synergistically inhibits cell growth and induces apoptosis in the ovarian epithelium. The mechanism(s) underlying the synergistic interaction of progestin and NSAIDs remains to be elucidated. It is possible that the synergistic outcome involves a convergence of apoptotic signaling events induced by the 2 agents or cooperation between 2 separate pathways to achieve enhanced apoptosis. Progesterone in particular is known to act via multiple...
signaling pathways, both receptor dependent and receptor independent. The mechanistic details of the synergy between NSAIDs and progestins could involve intricacies that can be investigated in a subsequent study of their respective molecular pathways.

In general, our normal ovarian epithelial cell culture and immortalized ovarian epithelial cell line were more likely to undergo apoptosis in response to progestins than to NSAIDs, whereas the converse was true in established ovarian cancer cell lines. The reason for this differential susceptibility to apoptosis is unclear. It is not surprising that the ovarian epithelium would be responsive to progesterone because the normal ovarian epithelium expresses progesterone receptor and progestin-related effects may play a normal housekeeping role related to the process of ovulation.

On the other hand, overexpression of cyclooxygenase-1/2 may be more typical in the dysplastic or malignant ovarian epithelium, making the more atypical ovarian epithelium more susceptible to induction of apoptosis by NSAIDs. This is similar to what has been described for other organ sites such as the lung, in which lung cancer cells have been shown to be more susceptible to apoptosis-including agents than benign lung epithelium. Regardless, when progestin and NSAIDs were combined in our study, marked susceptibility to apoptosis was observed in both the nonmalignant ovarian epithelial cell cultures as well as in ovarian cancer cell lines.

Similar to chemotherapy treatment of full-fledged cancers, it is likely that an optimal cancer preventive strategy would utilize a combinatorial chemopreventive strategy. This strategy could achieve enhanced cancer preventive potency, while minimizing the risk of failure because of dysplastic cells which are resistant to one or more preventive agents. Fortunately, compared with fully malignant cells, the dysplastic cells targeted by a chemopreventive approach are less likely to harbor multiple genetic alterations, decreasing the likelihood for resistance.

Importantly, if the combination of preventive agents chosen have both a synergistic effect on cancer preventive molecular pathways and nonoverlapping toxicities, it may be possible to achieve both increased cancer preventive efficacy with less overall toxicity, by reducing the dosage of each agent. Although progestins are generally well tolerated, their long-term use can have adverse effects on lipids and other factors related to cardiovascular health. Similarly, NSAIDs are well tolerated, but long-term use is associated with a risk of gastrointestinal bleeding and other cardiovascular related side effects.

The finding that progestins and NSAIDs have synergistic apoptotic effects in the ovarian epithelium suggests the possibility of a chemopreventive strategy using these agents combined in dosages and schedules that will minimize the side effects while hopefully yielding optimal ovarian cancer prevention.

REFERENCES


