

Feasibility of performing chemoprevention trials in women at elevated risk of ovarian carcinoma: Initial examination of celecoxib as a chemopreventive agent

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Abstract

Background. A pilot study was undertaken to determine the feasibility of examining a COX-2 inhibitor (Celecoxib) as a chemopreventive agent in women at increased risk of ovarian cancer undergoing risk reducing salpingoophorectomy.

Methods. Women at elevated inherited risk of ovarian carcinoma pursuing risk reducing salpingoophorectomy were eligible for this trial. Ten patients were assigned to a control group while 10 patients were administered Celecoxib (400 mg/day) for 3 months prior to surgery. Demographic data at enrollment was collected. Serum, urine, peritoneal fluid, and resected tissues were obtained for correlative laboratory study. Evaluation of serum VEGF alterations was examined using an ELISA-based assay.

Results. Enrollment of patients was completed in 16 months. Of 29 eligible patients, 20 enrolled onto the study. One patient from each group did not complete surgical intervention. No significant differences were observed in the enrollment characteristics between the groups. No occult cases of ovarian cancer were identified and no differences in the presence of follicular cyst, hemorrhagic cysts, or inclusion cysts were noted on initial pathologic review. While the mean serum VEGF levels obtained following the administration of a COX-2 inhibitor were lower than the pre-administration in 5 of 6 patients, statistical significance in this difference was not observed ($P = 0.359$). However, this is most likely due to the small number of serum samples available.

Conclusion. These results would suggest that chemoprevention trials in ovarian cancer will be eagerly embraced by this patient population. (Study supported by NIH 5P50CA83591.)

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Introduction

Approximately 70% of patients with a diagnosis of ovarian carcinoma will present as stage III or IV. Most patients will undergo an attempt at surgical debulking followed by 6 cycles of chemotherapy with a platinum-based regimen. This strategy will produce a complete clinical

response in 70% of patients [1]. However, the vast majority of these patients will experience a recurrence and the response to “salvage” treatments is often brief. These results are not surprising given that adjuvant therapy for any advanced stage solid tumors rarely yields durable cure rates. As such, treatment of ovarian carcinoma has yielded little improvement in long-term survival over the past 30 years [2].

Recognizing that therapeutic intervention of any advanced stage solid tumor is unlikely to produce a durable cure rate, attention has been given to screening to identify early stage disease amenable to curative resection. The

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fundamental flaw in this strategy centers upon the low prevalence of this disease. As such, the effectiveness of any screening strategy will be severely hindered by a low positive predictive value. Given an estimated prevalence of 50 cases per 100,000 population, a positive test with 99% specificity and 100% sensitivity would yield only one in 21 women with ovarian cancer [3]. The screening trials performed to date would support these ominous statistics. A representative study by Jacobs et al. utilized screening with CA-125 and ultrasound in 22,000 subjects [4]. These authors identified 41 women with positive screening results, of which 11 were noted to have cancer. Importantly, 70% of the identified cancers were still stage III or IV. Results such as these have led an NIH consensus conference to conclude that “There is no evidence available yet that the current screening modalities of CA-125 and transvaginal ultrasonography can be effectively used for widespread screening to reduce mortality from ovarian cancer...” [3].

Therefore, while the science of ovarian cancer therapeutics and screening strategies has evolved, little impact in long-term survival from ovarian cancer has been realized. Alternative strategies should, therefore, be considered. Cancer chemoprevention refers to the prevention of cancer with drugs or natural substances. Increasing knowledge of inheritable genetic lesions in cohorts of patients allows for the identification of high-risk populations that would potentially benefit from chemopreventive interventions. Moreover, a carcinogenic pathway has been suggested which involves uninterrupted ovulation in a growth stimulating hormonal milieu leading to increased probability of genetic lesions and expansion of tumorigenic clones [5]. Increased knowledge of molecular alterations occurring along this pathway affords opportunities for focused interventions. Current evidence of the potential for the prevention of ovarian cancer lies in epidemiological data. These data strongly support a protective role of oral contraceptives against the development of ovarian carcinoma [6]. Risk reducing oophorectomy, tubal ligation, and hysterectomy have also been shown to be protective [6].

Chemopreventive agents should be associated with low toxicity and ease of administration, given the relative state of health of patients pursuing preventive measures. Moreover, as ovarian carcinoma is a relatively rare cancer, ideal agents would also prevent other more common cancers the patient might be at risk for such as colon or breast cancer. Preventive agents in ovarian cancer should also be free of potential stimulatory effects on other cancer for which the patient may be at risk, such as breast cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) have generated significant enthusiasm as chemoprevention agents, particularly in the area of colon carcinoma [7]. Moreover, preliminary data exists to suggest that these agents may also exhibit preventive effects in a variety of other malignancies which impact public health [8–11]. COX-2 inhibitors are a class of NSAID selective for the COX-2 enzyme, whereby significant upper gastrointestinal toxicity is averted when administered orally,

making it an attractive potential chemopreventive agent. Finally, NSAIDs represent a non-hormonal agent posing little risk of breast cancer. To more directly address the potential preventive role of NSAIDs, observational studies of ovarian carcinoma have suggested a risk reduction with the use of some NSAID derivatives [12–15].

A strong rationale, therefore, exists for the study of chemoprevention strategies utilizing derivatives of non-steroidal anti-inflammatory drugs in the context of ovarian carcinoma. To date, however, no clinical trial utilizing any chemopreventive agent in the context of patients at elevated risk of ovarian carcinoma has been completed in the United States. We therefore undertook this trial to determine the feasibility of examining a COX-2 inhibitor (Celecoxib) as a chemopreventive agent in women at increased risk of ovarian cancer undergoing risk reducing salpingoophorectomy.

Methods

Subject participation

Women at elevated inherited risk of ovarian carcinoma who had decided to pursue risk reducing salpingoophorectomy were eligible for this trial. Briefly, women greater than age 35 who are BRCA1/2 positive or who, by pedigree, would have at least a 10% risk (by BRCAPRO Computerized Risk Program) of having a BRCA mutation were eligible. At a minimum, eligible subjects were noted to have two first or second degree relatives affected with breast or ovarian cancer where at least one relative was affected at age less than 50.

After enrolling onto the study, patients were assigned to the Celecoxib group or control group in cohorts of five serial patients. This was done to allow for placement of patients into the Celecoxib group should they present with a history of arthritis requiring this medication for relief. However, this safeguard was not utilized, as, over the course of the enrollment period, a single patient presented on Celecoxib and was enrolled during a period where patients were being assigned to the Celecoxib group.

Ten patients were assigned to a control group while 10 patients were administered the COX-2 inhibitor, Celecoxib (400 mg/day), for 3 months prior to surgery. The dose of Celecoxib was selected as it is the maximum FDA approved dose for use in alternative indications. Demographic data was collected at enrollment. Patients assigned to receive the COX-2 inhibitor presented for a midpoint evaluation to exclude toxicity at 1.5 months. Blood, urine, peritoneal fluid, and resected tissues were subsequently obtained for correlative laboratory study using histologic morphology, ELISA analysis for Vascular Endothelial Growth Factor (VEGF) levels. This study was performed and funded under the auspices of the University of Alabama at Birmingham Specialized Program of Research Excellence (SPORE) in Ovarian Cancer (NIH 5P50CA83591).

Prior to institution of accrual, the protocol and consent were reviewed by the Institutional Review Board of University of Alabama at Birmingham and appropriate approvals were obtained. All patients enrolled onto the study provided signed informed consent.

Surgical procedure

At a minimum, all patients underwent complete resection of the bilateral adnexa inclusive of the fallopian tube and ovary. At the time of surgery, the entire peritoneal cavity was examined either by gross examination at laparotomy or by laparoscopic evaluation. Specifically, this included examination of all peritoneal surfaces, the bilateral diaphragmatic surfaces, the mesentery of the small intestine, the small and large intestines, omentum, the pelvic and bladder peritoneum, the cul de sac, and the uterine serosa when present. Abdominal washings were obtained for cytologic evaluation. The complete adnexa were removed inclusive of the ovary and fallopian tube, a 1 cm portion of peritoneum to the lateral and medial sides of the ovary/fallopian tube complex, a 2 cm margin of infundibulopelvic ligament proximal to the ovary, and a 1 cm margin of the ovarian ligament distal to the ovary when the uterus was retained. A majority of patients were managed with minimally invasive techniques.

Histologic evaluation of resected tissues

To insure minimal risk that an occult ovarian cancer would be unidentified, the entire ovary and fallopian tube were submitted for pathologic review. Specifically, the ovary and fallopian tube samples were fixed in 10% buffered formalin, processed in a routine fashion, and embedded in

semi-synthetic tissue. Five micrometer tissue sections were obtained and stained with hematoxylin and eosin. These sections were then evaluated microscopically and reported on the pathology form (Fig. 1).

Serum VEGF assay

Initial serum samples were collected from seven women who were considered high risk for ovarian cancer. The patients were treated with a COX-2 inhibitor, and 2 months later, another serum sample was collected. One patient dropped out of the study, two patients did not return for a second serum sample, and in one patient, only a plasma sample was obtained, leaving a total of six pre- and post-COX-2 inhibitor administration samples available for study. All of the serum samples were stored at -125°C by the University of Alabama at Birmingham Tissue Procurement core facility. Serum VEGF concentrations were measured using a sandwich enzyme immunoassay (Quantikine Human VEGF Immunoassay; R&D Systems Inc., Minneapolis, MN) as indicated by the manufacturer's instructions.

First, 100 μl of a buffered protein base was added to each well on the microplate strips. Each microplate strip contained bound monoclonal antibodies for VEGF. Quality controls, standards, and subject serum samples were added, 100 μl each, to individual wells in triplicate. The plate was incubated for 2 h at room temperature, allowing binding of any VEGF present to the antibody precoated on the plate. The wells were washed multiple times to remove any unbound substances. Next, 200 μl of VEGF conjugate, a polyclonal antibody specific for VEGF conjugated to horseradish peroxidase, was added to each well and the plate was incubated at room temperature for 2 h. The wells were washed to remove

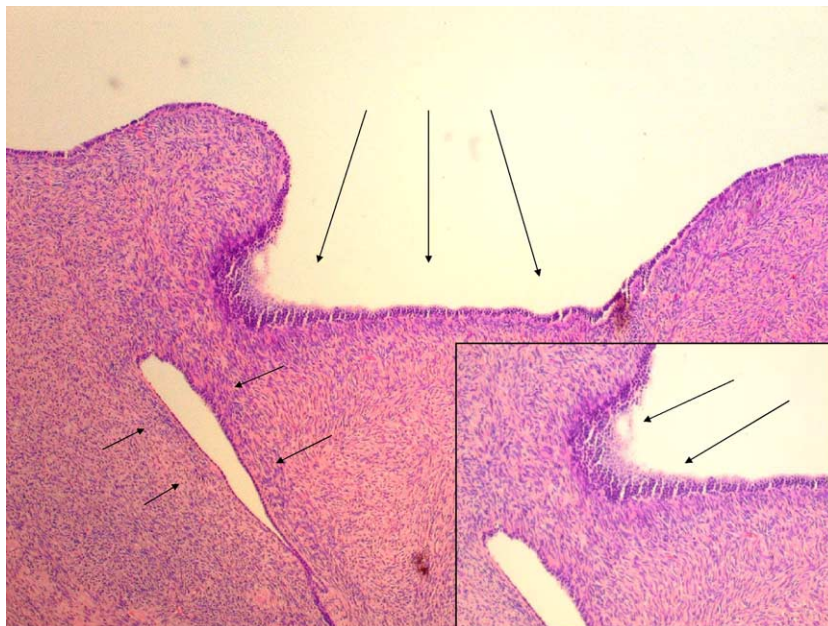


Fig. 1. Hematoxylin and eosin stain of a representative histologic section of ovarian tissue demonstrating intact epithelial cells amenable to further study utilizing immunohistochemical assays.

Table 1
Premenopausal ovarian and breast cancers occurring in pedigrees of study population

Age	Pt HX	1st Pre-Br	1st Pre-OV	2nd Pre-Br	2nd Pre-OV	3rd Pre-OV
42					×1	
40					×1	
51	Br	×2				
47	Br	×1	×1	×2		
38					×3	
38		×3	×1	×2		
46			×1			
40					×1	×2
53			×1			
49	Br	×3		×1		
42				×1		
56		×2			×1	
59		×1			×1	
55	Br	×3		×1		
39	Br	×1		×2		
54	Br					
55				×1		
37			×2		×1	

All patients have at least one additional postmenopausal family member with history of breast or ovarian cancer.

unbound substances and 200 µl of a substrate solution, a combination of equal parts hydrogen peroxide and chromogen, was added to each well. The plate was incubated for 25 min at room temperature. The substrate solution produced a color change proportional to the concentration of VEGF bound to the wells during the first step of the assay. A stop solution, sulfuric acid, was added (50 µl) to stop the color development. Intensity was then determined using a microplate reader set to 450 nm (µQuant; Bio-tek Instruments, Inc., Winooski, VT). Finally, a standard curve was created by plotting the mean absorbance of each standard. Mean serum levels of VEGF were determined prior to initiating a COX-2 inhibitor and were compared to mean serum VEGF levels obtained 1.5 months of administration for 6 patients where pre- and post-Celecoxib group serum samples were available. Analysis of one way variance (ANOVA) was used as a test of significance with an alpha level equal to 0.05.

Results

While the protocol allowed for a 24-month accrual period, the enrollment of patients was completed in 16 months. Of 29 eligible patients, 20 (69%) enrolled onto the study. One patient from each group did not complete surgical intervention. Only one of the 41 patients screened for this study was non-Caucasian, while all patients who subsequently enrolled onto this study were Caucasian.

No differences were observed in the enrollment characteristics between the groups in regard to mean age (Celecoxib group—46.3 vs. control—46.8), parity (Celecoxib group—1.4 vs. control—2), personal history of breast cancer (Celecoxib group—4 vs. control—3), or mean baseline

serum CA125 level (Celecoxib group—11.4 vs. control—14.0). One patient in the Celecoxib group used Celecoxib prior to enrollment while one patient in the control group had a history of aspirin use. When examining the family pedigrees, all patients had, at a minimum, either a first or second degree relative with a history of breast or ovarian cancer where one of the cancers occurred at age less than 50 (Table 1). Six patients had a personal history of breast carcinoma and a majority of patients also had a first degree relative with a history of breast or ovarian cancer occurring in the pre-menopausal state.

Eighteen of 20 patients completed the study and underwent a risk reducing surgical procedure. The majority of patients (12 of 18 patients) underwent procedures that incorporated minimally invasive techniques. During the initial inspection of the abdominal cavity, no cases of advanced ovarian carcinoma were identified. As noted in the Methods section, the resected ovaries and tubes were submitted completely for pathologic examination to exclude occult malignancies. No cases of occult carcinoma of the ovary or fallopian tube were identified. None of the cytology specimens were positive for malignant cells. The majority of patients enrolled onto study did have follicular or hemorrhagic cysts present on pathologic examination, and one benign serous cystadenoma was identified.

Our initial evaluation of a potential biomarker alteration has centered on an investigation of the COX-2 inhibitor's ability to influence serum VEGF levels. Six matched serum specimens representative of pre-COX-2 inhibitor administration and post-COX-2 inhibitor administration (obtained at the 1.5 month midpoint evaluations) were available for analysis of serum VEGF levels. In five of six patients, a decrease in serum VEGF levels was observed following 1.5 months of administration of an FDA-approved dose of Celecoxib (Table 2). While the mean serum VEGF levels obtained following the administration of a COX-2 inhibitor were lower than pre-administration in 5 of 6 patients, statistical significance in this difference was not observed ($P = 0.359$). This trend should also be interpreted with caution as potential fluctuations in serum VEGF levels may be due to alternative causes such as cyclic alterations during other physiologic cycles such as the menstrual cycle.

Table 2
Serum VEGF levels (pg/ml) in serum specimens obtained from 6 patients prior to the initiation of Celecoxib (400 mg/day) and a subsequent serum specimen obtained after 1.5 months of administration

Study subject	Pre-Celecoxib	Post-Celecoxib
1	130.7	100.9
2	436.8	285.6
3	454.4	574.1
4	343.8	216.9
5	126.6	82.8
6	312.7	298.7

Serum VEGF assay run in triplicate for each specimen.

Discussion

In this study, we demonstrated the feasibility of conducting a controlled clinical trial of a chemopreventive agent in subjects at elevated inherited risk of ovarian cancer. The subjects were accrued to this trial within the first 16 months of a planned 24-month enrollment period. A high percentage of patients (90%) completed the study and underwent risk reducing surgery, with a majority of procedures incorporating minimally invasive techniques. The chemopreventive agent utilized, a COX-2 inhibitor, was well tolerated and the length of administration did not appear to adversely influence the ability of enrolled subjects to complete the trial. Importantly, the finding that serum levels of VEGF may be decreased by chronic administration suggests that a biomarker can be altered by the COX-2 inhibitor and can facilitate power analysis of future studies.

To our knowledge, this study represents the first successful completion of a controlled trial of a chemoprevention agent in a population at risk for ovarian carcinoma in the United States. Potential barriers to the completion of these types of trials have been suggested and include scarcity of the patient population, duration of administration of the agent, toxicity of the chemoprevention agent, and potential for a given chemoprevention agent to induce alternative related cancers (i.e. breast carcinoma). Many of our subjects were also participating in the multicenter ROCA ovarian cancer screening trial. As a result, we purposefully maintained similar eligibility criteria to both trials. In effect, we assumed that some of the women undergoing screening would desire risk reducing surgery during the course of screening. Moreover, we found these women motivated to participate in a trial of chemopreventive agents that in all likelihood (due to the brief administration period) would be of more value to others than themselves. A weakness in our subject accrual is the lack of individuals representing underserved populations. Examination of barriers to trial entry in underserved populations needs to be examined and may reflect a lack of awareness of the existence of such studies, lack of awareness of risk factors predisposing to ovarian carcinoma skepticism in regard to participating in these trials, or the absence of health insurance that would allow financial coverage of the expensive risk reducing surgical procedure.

The COX-2 inhibitors are an attractive agent for future study as a potential chemopreventive agent for ovarian cancer in high-risk populations. Observational studies have suggested a risk reduction of ovarian carcinoma in subjects who take NSAIDs on a regular basis [12–15]. In addition, similar evidence supports a potential preventive effect of malignancies of the lung, gastrointestinal tract, cervix, colon and breast [8–11]. The ability of this class of agents to potentially act as a chemopreventive agent in breast carcinoma deserves special mention, as breast cancer commonly occurs in this risk cohort. Moreover, Harris et al. have recently reported data from the WHI study where

regular NSAID use produced a 21–28% reduction in the risk of subsequent development of breast carcinoma [16]. Ideally, a chosen chemopreventive agent should pose no threat to increasing the risk of an associated malignancy in a high-risk population. There is no evidence in high-risk populations that the COX-2 inhibitors enhance the risk of breast cancers in a population at risk for inherited breast/ovarian cancer syndromes. In addition, we found 3 months of administration of a COX-2 inhibitor to be well tolerated and associated with minimal toxicity. Patients taking the COX-2 inhibitor in this trial presented for a midpoint evaluation where no subjective toxicity was identified, nor was there evidence of renal compromise by elevation of serum creatinine.

These findings should, however, be seen in light of recent cardiac toxicity observed in COX-2 specific inhibitor class of NSAIDs leading to withdrawal from market of several agents [17,18]. Given the healthy status of most subjects pursuing preventive strategies of ovarian cancer, some consideration should be given to alternative NSAIDs such as aspirin. Acetylsalicylic acid (aspirin) is an NSAID with a long track record of safety in general use. As noted in the “Physician’s Desk Reference”, aspirin is indicated for the prevention of ischemic stroke, transient ischemic attack, and recurrent myocardial infarction. Laboratory evidence suggests that the primary mechanism of action is acetylation of cyclooxygenase 2 (COX-2), thereby inhibiting the activity of this enzyme [19–21]. Aspirin is also recognized as a non-specific inhibitor of COX-1 enzyme resulting in potential risk for erosive gastritis, which would need to be considered during chronic administration. As a chemopreventive agent, aspirin (and its derivatives of indomethacin and COX-2 inhibitors) has been demonstrated to exhibit protection against ovarian cancer in epidemiological studies, interfere with normal processes of ovulation, and inhibit downstream inflammatory mediators suggested to play a role in carcinogenesis. Aspirin has been prospectively tested as a potential preventive agent in other cancer sites, primarily in subjects at risk for the development of colorectal carcinoma [22,23]. Finally, Markov models of hypothetical cohorts comparing aspirin to COX-2 inhibitors for the prevention of colorectal carcinoma suggests improved cost effectiveness, decreased morbidity, and decreased mortality in favor of use of aspirin [24]. Aspirin, therefore, may represent a low-cost alternative to specific COX-2 inhibitors with a more optimal cardiovascular toxicity profile.

Within the framework of a chemoprevention trial, a desirable endpoint is the influence of a chemopreventive agent on a molecular biomarker. The utilization of biomarkers is even more important in the context of chemoprevention trials of ovarian cancer. It is highly unlikely that a prevention trial could be accomplished with the development of ovarian cancer as the primary endpoint due to the low incidence of these cancers even in high-risk populations. In this study, we examined the influence of the

administration of COX-2 inhibitors on a serum biomarker, vascular endothelial growth factor or VEGF. The study of serum biomarkers offers several advantages over direct assessment of the end organ. Acquisition of samples for serial analysis poses minimal risk to the patient. The identification of an alteration of the serum biomarker allows for the development of specific hypothesis that can be tested using ultra-sensitive techniques at the end-organ (i.e. ovary) such as gene array technology. When examining the end organ, more comprehensive studies of various molecular pathways related to the serum biomarker can be developed. Finally, the identification of an altered molecular marker in the serum is generalizable to other target end-organs (i.e. breast and colon) where a selected agent may play a potential role in prevention. Ultimately, however, it remains important that alterations in the serum biomarker be confirmed to produce desirable effects in the end organ and these studies will be pursued with specimens banked in this trial.

Serum VEGF is an interesting serum biomarker to analyze. The finding that serum VEGF levels decreased following the administration of a COX-2 inhibitor in five of six patients is supported by Csiki et al. following the administration of Celecoxib (400 mg BID) in patients with non-small cell lung cancer receiving Docetaxel (personal communication). The results from 43 subjects as presented in abstract form at the 11th SPORE Investigators workshop demonstrated a 45% reduction in serum VEGF levels following the administration of the COX-2 inhibitor ($P = 0.0005$). Laboratory investigations have also confirmed relationships between COX-2 inhibition and VEGF expression. In vitro studies by Leung et al. demonstrated that transfection of gastric carcinoma cell lines with a COX-2-expressing vector resulted in increased production of prostaglandin E2 and VEGF [25]. Yoshida et al. reported in vivo results from a ddy mouse model of sponge angiogenesis using Lewis Lung Carcinoma cells [26]. These investigators demonstrated suppression of VEGF expression and angiogenesis when the mice were administered the selective COX-2 inhibitor NS-398. Additionally, in a murine model of liver metastases using SCID mice and HT-29 colon carcinoma cell lines, Yamauchi et al. reported that the administration of the selective COX-2 inhibitor JTE-522 suppressed VEGF expression and prevented liver metastases [27].

More specific to ovarian function, VEGF appears to play a role in follicular development. Zimmerman et al. used VEGF neutralizing antibodies to inhibit angiogenesis-dependent follicular development, which was independent of gonadotropin stimulation [28]. Findings such as these have led Geva and Jaffe to postulate that VEGF and vascular permeability factor (VPF) play an important role in the cyclic growth of ovarian follicles and corpus luteum development [29]. Additionally, VEGF expression appears to be induced by both FSH and LH/hCG receptor activated pathways. These laboratory postulates take on potential

clinical significance as the NSAID, indomethacin, has been observed to interfere with normal ovulation and produce a syndrome of unruptured leutenized follicles in reproductive-age females [30]. Similarly, Pall et al. have reported a randomized trial of the administration of the COX-2 inhibitor, rofecoxib, where the induction of delayed follicular rupture was observed when compared to a placebo group [31]. In addition to the inhibition of ovulation, Altinoz and Korkmaz have proposed that a mechanism of ovarian cancer prevention is a shared pathway dependent on the suppression of NF Kappa B activity resulting in a down-regulation of inflammatory mediators such as COX-2, VEGF, IL-8 and uPA [32]. In regard to VEGF, aspirin has been demonstrated to inhibit colon cancer medium-induced endothelial tube formation associated with decreased VEGF expression [33]. Specific COX-2 inhibition, using Loxoprofen, suppressed both serum and intratumoral VEGF concentrations in Lewis lung cancer cell-implanted mice [34].

The findings from this study support further clinical investigations into potential chemopreventive agents for ovarian carcinoma. Controlled clinical trials in a cohort at elevated risk for ovarian cancer desiring risk reducing surgery appear feasible, and tissues can be collected for corroborative laboratory investigation. The finding that COX-2 inhibitors potentially influence serum VEGF should be further examined in a larger trial of a high-risk cohort and the potential downstream consequences of decreased serum VEGF levels on molecular pathways within the ovary should be pursued.

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