

AD_____

Award Number: W81XWH-04-1-0322

TITLE: A Controlled Trial of Chemoprevention Using COX-2 Inhibitors in an Avian Model of Spontaneous Ovarian Carcinogenesis

PRINCIPAL INVESTIGATOR: Mack N. Barnes, M.D.

CONTRACTING ORGANIZATION: University of Alabama at Birmingham
Birmingham, AL 35294-0109

REPORT DATE: September 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 01-09-2007		2. REPORT TYPE Final		3. DATES COVERED (From - To) 15 FEB 2004 - 14 AUG 2007	
4. TITLE AND SUBTITLE A Controlled Trial of Chemoprevention Using COX-2 Inhibitors in an Avian Model of Spontaneous Ovarian Carcinogenesis				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-04-1-0322	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Mack N. Barnes, M.D. E-Mail: mbarnes@uabmc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Alabama at Birmingham Birmingham, AL 35294-0109				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The objective of this study was to determine, in a controlled chemoprevention trial, the ability of a COX-2 inhibitor to inhibit the development of spontaneously arising genital tract adenocarcinoma in the laying hen (<i>Gallus Domesticus</i>) animal model of ovarian cancer. Following a dose finding study for the COX-2 inhibitor, Rimadyl, 480 hens were utilized in a controlled trial of this agent to determine the subsequent development of genital tract adenocarcinoma by histologic examination. Assessment of egg count data suggests no reduction in the number of eggs produced in the animals treated exposed to COX-2 inhibitor. There was no evidence of a reduction of reproductive tract adenocarcinoma. In this study, no reduction in the incidence of genital tract adenocarcinoma was observed in the laying hen model following exposure to a COX-2 inhibitor. It is of significant interest that no effect on ovulatory activity was observed. This study potentially helps shed light on the importance of reducing ovulatory activity in the pursuit of ideal chemopreventive agents for ovarian cancer.					
15. SUBJECT TERMS Ovarian cancer, chemoprevention, COX-2 inhibitor, ovulation					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	43	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	6
Key Research Accomplishments.....	10
Reportable Outcomes.....	11
Conclusion.....	14
References.....	17
Appendices.....	21
Supporting Data.....	21

ANNUAL (FINAL) REPORT: CONTROLLED TRIAL OF CHEMOPREVENTION USING COX-2 INHIBITORS IN AN AVIAN MODEL OF SPONTANEOUS OVARIAN CARCINOGENESIS (W81XWH-04-1-0322)

INVESTIGATORS: Mack N. Barnes MD, Wallace D. Berry PhD

INTRODUCTION

Ovarian carcinoma is the fourth leading cause of cancer death in the female population and the most fatal gynecologic malignancy (1). Approximately, 75% of the cases of ovarian cancer present in an advanced stage where therapy consists of surgical debulking and adjuvant chemotherapy (1). Like many other disseminated solid tumors, studies to date that center on therapeutic strategies suggest it is unlikely that current treatment of advanced ovarian carcinoma will yield significant improvements in long term mortality from this insidious disease.

Chemoprevention strategies may provide a rational alternative approach for meaningful reductions in deaths attributable to ovarian carcinoma (2). Cancer chemoprevention refers to the prevention of cancer with drugs or natural substances which do not cause significant side effects when used chronically. Chemoprevention strategies may provide a rational alternative approach for meaningful reductions in deaths attributable to ovarian carcinoma (2). Non-steroidal anti-inflammatory drugs (NSAIDs) have generated significant enthusiasm as chemoprevention agents, particularly in the area of colon carcinoma (3). Moreover, preliminary data exists to suggest that these agents may also exhibit preventive effects in cancers of the breast, lung, cervix, and other components of the gastrointestinal tract (4-7). 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 (8-11). These studies are difficult to interpret due to the retrospective nature of the study design, the potential for recall bias, and the possibility that the protective effect of these agents might be blunted as they are not taken on a daily basis in the same way oral contraceptives are. However, a reduction in the risk of ovarian cancer of approximately 50% has been observed in some of these studies (10,11).

While a strong rationale for chemoprevention of ovarian carcinoma exists, a mechanism for the comprehensive evaluation of novel compounds is impeded by the lack of a validated animal model of induced or spontaneous ovarian carcinogenesis. Identification of spontaneous reproductive tract adenocarcinoma in the laying hen (*Gallus Domesticus*) may provide one answer to this dilemma (12-14). An evaluation of 200 two-year old hens documented a 4% rate of grossly identifiable reproductive tract adenocarcinomas with a histologic appearance similar to human epithelial ovarian cancers (14). Alfonso et al, demonstrated a 45% rate of ovarian and oviductal adenocarcinoma in 4 year old hens suggesting a higher incidence of cancer development as age increases (15).

Therefore, the purpose of this study was to explore the hypothesis that administration of the potential class of chemopreventive agents, COX-2 inhibitors, will result in a decreased rate of development of ovarian carcinoma in an avian model of spontaneous ovarian carcinogenesis. Two primary objectives were to be examined in

the course of this study. The first objective was to determine a tolerable daily dose (mg/kg) of COX-2 inhibitor, admixed with standard feed, in the laying hen. The effect of varying doses of COX-2 inhibitor on egg-laying activity as a surrogate marker for ovulatory frequency was assessed. The dose identified was then used in the controlled trial. The second primary objective was to determine, in a controlled chemoprevention trial, the ability of a COX-2 inhibitor to inhibit the development of spontaneously arising genital tract adenocarcinoma in the laying hen.

BODY

The stated objectives that were submitted in the original proposal were completed in accordance with the time line proposed in this funding mechanism. The objectives were completed using the following methodology.

Methods

COX-2 Inhibitor (Rimadyl) Dose Finding in the Avian Hen

Preliminary unpublished studies had suggested significant and lethal toxicity with the NSAID, indomethacin, when administered to laying hens (personal communication: W. Berry). In addition, there is a paucity of information regarding the use of COX-2 inhibitors in the avian hen. Rimadyl (Pharmacia Inc.) is a COX-2 specific inhibitor approved for use in veterinary medicine and available in a stable powder form. Once identified, the daily tolerated dose would then be used in the controlled trial.

Three dose levels were established as indicated (Table 1).

<u>Cohort</u>	<u>Dose of COX-2 Inhibitor</u>
Control:	0 mg/kg body weight
Dose 1:	2.5 mg/kg body weight
Dose 2:	5 mg/kg body weight
Dose 3:	10 mg/kg body weight

Rimadyl was added with other feed ingredients at the time feed was mixed in the feed mill and delivered to the hens. 5 hens were utilized per dose level for a total of 20 hens. The hens were monitored for egg laying activity as defined by percent egg laying activity control (0 mg/kg) and egg shell quality. In addition, toxicity was monitored based on general appearance, feather quality, evidence of gastrointestinal bleeding, and death. The planned dose for use in the study would be identified as the dose not associated with the aforementioned untoward toxicity.

Avian Study Population

Prior to initiation of the study, a power calculation was performed assuming a 40% incidence of genital tract adenocarcinoma. It was estimated that to identify a reduction in disease of 20% at the 0.05 level of significance a treatment group of 88 hens and a control group of 88 hens would be required. The informative cases were defined as those hens completing at least six months of a course of treatment. Hens that were living at the completion of the study period were sacrificed and included in the “informative group”. Significant attrition from non-malignant causes is known to occur as hens age, particularly past two years. Therefore, to initiate this study 480 hens (*Gallus Domesticus*) were acquired 8/3/04 (kindly provided by D Roland PhD, Professor of Nutrition, Auburn University). The breed of hens was designated as *Gallus Domesticus* and were two years of age upon study initiation.

In order to account for natural attrition, we established a treatment group (240 hens) and a control group (240 hens). It is our practice to allow a period of time for hens to “acclimate” and, therefore, a period of 8 weeks was utilized to allow the hens to acclimate. The controlled study was then initiated November 19, 2004, using a daily

dose of Rimadyl of 10mg/kg body weight. A subjective increase in number of hens that were dying was recognized as they aged and, therefore, the remaining live hens were sacrificed and necropsy performed September 29, 2006. During the study period, animals were housed in animal care facilities provided by Auburn University School of Poultry Science under the direct supervision of one of the studies co-authors (W.D.B.). The chickens were maintained and sacrificed according to standards governing animal investigations and provided by Institutional Animal Care and Use Committee at Auburn University. After the study period had elapsed, the animals were euthanized in accordance with the rules established by the Panel on Euthanasia of the American Veterinary Medical Association listed in the 1993 Report of the American Veterinary Medical Association Panel on Euthanasia. Additionally, in January of each year an “induced molt” was performed. This procedure entails limiting food and light until a 25% reduction in body weight from baseline is achieved in the hen. Induced molts restore normal egg laying activity in this species of captive fowl and is performed as part of the routine care of these animals irrespective of the ongoing study. If not performed, the egg laying/ovulatory activity of the control group would cease. In order to maintain a “standardized environment” both control and treated hens were subjected to annual induced molts.

At the time of necropsy, the presence or absence of ascites and carcinomatosis was noted. Additionally, at the time of necropsy, samples of ovary/oviduct were obtained if no gross evidence of cancer was present. If gross evidence of cancer was identified, a sample of the metastatic tumor as well as the ovary/oviduct were obtained. Only those cases where cancer involved the ovary/oviduct structure primarily (with or

without the presence of metastatic disease) are reported as index cases of reproductive tract adenocarcinoma.

Monitoring of Egg-laying activity

Periodic monitoring of egg-laying activity in the respective study groups was performed. To assess egg laying activity, a cohort of control hens and “treated” hens were monitored on a weekly basis throughout the 24 month study period and egg production totaled. More specifically, egg laying activity was monitored in available hens from week 1 to week 97. The egg production rate of the two groups could then be compared to assess the effect of administration of COX-2 inhibitor on egg laying activity and an association with the development of genital tract adenocarcinoma.

Histologic Evaluation

Tissue specimens were fixed in neutral-buffered formalin and processed to paraffin blocks. The specimens were then taken to the UAB Ovarian Cancer Core pathology facility for further processing. The specimens were paraffin embedded and five-micron sections were cut from each paraffin block, stained with hematoxylin and eosin (H&E), and reviewed by a pathologist (W.E.G.) for presence or absence of reproductive tract adenocarcinoma. All specimens were reviewed by a single pathologist (W.E.G.) who had gained experience in the histopathology of avian reproductive tract adenocarcinomas during a previous study (14). As all cases had oviductal tissues available for histologic review, cases were subjectively divided into small, medium, and large volume of tumor for a more detailed analysis.

Statistical Analysis

A qualitative assessment of the influence of administration of the preventive agent, Rimadyl (COX-2 inhibitor) on egg laying activity was depicted graphically. The prevalence of reproductive tract adenocarcinoma was determined after histologic examination of material from the index case hens. The rate of cancer in the treatment group (148 informative cases) was then compared to the control group (156 informative cases). A risk ratio for the presence of reproductive tract adenocarcinoma and a 95% confidence interval calculated were to be calculated if a difference was observed.

KEY RESEARCH ACCOMPLISHMENTS

- Dose Finding Study completed 10/04
- No toxicity demonstrated for Rimadyl (COX-2 inhibitor) at 10mg/kg body weight and this dose selected for treatment group
- Hens acquired for trial and acclimated (8/04-10/04)
- Controlled trial initiated 11/04. 240 hens were allotted to a treatment group while 240 hens were allotted to a control group (in accordance with proposed statistical parameters).
- Treatment of hens terminated in 9/06 and remaining hens euthanized and necropsy performed with tissues transported to University of Alabama at Birmingham (Laboratory of W Grizzle).
- Tissues analyzed histologically and determination made of potential differences in incidence of genital tract adenocarcinoma completed in accordance with time line 3/07

- Results evaluated and initial draft of manuscript developed and DOD report submitted 9/06 in accordance with no cost extension granted.

REPORTABLE OUTCOMES

The reportable outcomes obtained from this study are outlined as follows. The initial dose finding study was completed at four dose levels of Rimadyl as follows; 0 mg/kg body weight (control), 0.25 mg/kg body weight, 5mg/kg body weight, and 10mg/kg body weight. Over an 8 week study period (8/04-10/04), no toxicity and no lethal events were noted. In addition, there did not appear to be any effect on ovulatory activity, even at the highest dose level. Egg laying activity was also monitored over the course of the 2 year study period. Given, the ability of these hens to tolerate the highest dose level, 10mg/kg body weight was determined to be the dose that would be utilized in the controlled trial.

A total of 480 laying hens were initially divided into a treatment and control group consisting of 240 hens each. During, and upon completion of the second year of exposure to COX-2 inhibitor, 304 hens were subjected to necropsy and represent the “informative” index cases as these were felt to be adequately exposed to the intervention. For analysis of “informative subjects,” 156 hens constituted the control group and 148 hens constituted the treatment group. The 176 hens not included in the “informative cases” were those not exposed to adequate COX-2 inhibition, hens that expired and were culled during “weekend cage maintenance”, and some that were not evaluated due to poor tissue depending on cause of death. These hens did not undergo histologic examination.

Assessment of egg count data suggests no reduction in the number of eggs produced in the animals treated exposed to COX-2 inhibitor when compared to control (Table 2).

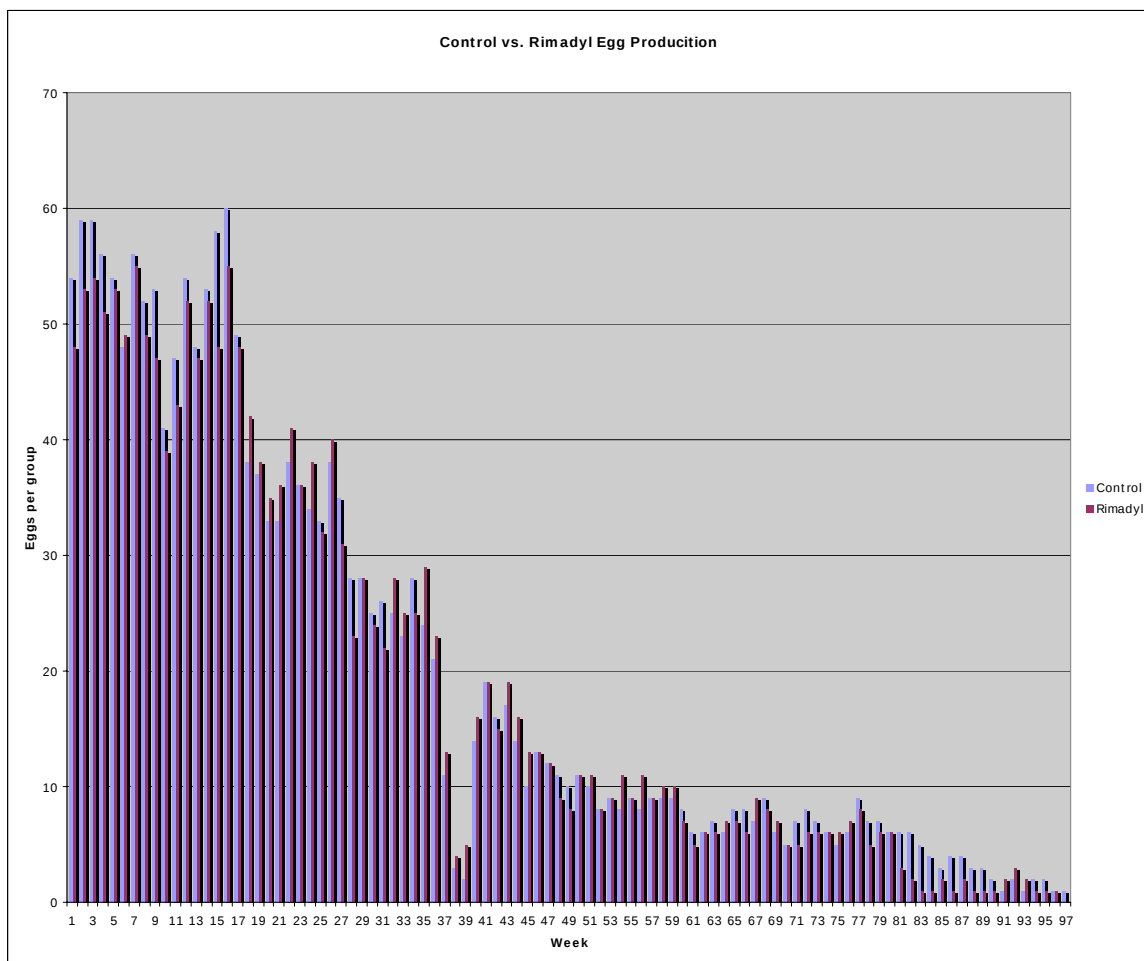


Table 2. Egg Laying Production of COX-2 Exposed Hens Compared To Control Hens During Week 1 Through 97 Of The Study Period.

As noted previously, no difference in egg-laying activity was observed during the brief dose finding study. An overall decline in egg laying activity is observed in the entire cohort of animals as they aged into their fourth year of life, and this physiologic phenomenon has been observed in prior studies and does not appear to be influenced

by administration of a COX-2 inhibitor. A notable decrease in egg production is observed during week 37-39, which is due to the aforementioned induced molt.

Tissue specimens from all 304 of the index (or informative) cases were examined histologically for the presence or absence of reproductive tract adenocarcinoma. Consistent with a previous report, multiple cases of histologically confirmed spontaneously arising reproductive tract adenocarcinomas were identified (12-15).

The overall rate of spontaneously arising reproductive tract adenocarcinomas were observed in the study cohort of 304 four year-old hens was 64% (genital tract adenocarcinoma observed in 194 out of 304 hens). Of the tissues examined from the informative cases, there was no evidence of a reduction of reproductive tract adenocarcinoma. In aggregate, 98 of 156 (62%) hens in the control group were noted to have histologic evidence of genital tract adenocarcinoma while 96 of 148 (64%) in the Rimadyl group had histologic evidence of genital tract adenocarcinoma. These results were not significantly different. A subgroup analysis was then performed to see if any differences were present in volume of tumor present, and as Table 3 indicates no significant differences were observed.

<u>Volume of Tumor</u>	<u>Control</u>	<u>Rimadyl</u>	<u>Difference</u>
No Tumor	58	52	n.s.
Small	13	17	n.s.
Moderate	23	15	n.s.
Large	62	64	n.s.

Table 3. Evaluation Of Volume Of Tumor Assessed In Hens Exposed to Rimadyl Versus Control.

CONCLUSIONS

The ability of chemopreventive strategies to impact the incidence and subsequent mortality rate of ovarian carcinoma has been a neglected field of study. However, renewed interest in prevention of ovarian cancer is surfacing given the minimal impact on long term survival of therapeutic strategies for advanced disease. Screening strategies for ovarian cancer also have been tested but, at present, there is little evidence for the success of this strategy given the prohibitively low prevalence of disease (16). Therefore, prevention of ovarian cancer may represent the most rational investigational strategy to obtain a reduction in death from disease.

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 (3). Moreover, preliminary data exists to suggest that these agents may also exhibit preventive effects in cancers of the breast, lung, cervix, and other components of the gastrointestinal tract (4-7). 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.

Intriguing studies have demonstrated an anti-proliferative effect of NSAIDs in ovarian cancer and other solid tumors. Indeed, a randomized trial comparing

indomethacin to placebo in end stage patients with solid tumors revealed a surprising survival advantage of 510 days compared to 250 days ($p < 0.05$) in patients with metastatic cancer (17). Recent reports have also documented the ability of NSAIDs, and COX-2 inhibitors specifically, to inhibit ovarian tumor cell growth and result in apoptosis in primary ovarian cancer cell cultures (18). While these studies are provocative, they more strongly address the role of these agents as anti-neoplastic drugs rather than agents that either prevent the development of cancer or cause reversion of the malignant phenotype.

To more directly address the potential preventive role of NSAIDs, case-control studies of ovarian carcinoma have suggested a risk reduction with the use of some NSAID derivatives. (8-11). These studies are difficult to interpret due to the retrospective nature of the study design the potential for recall bias, and the possibility that the protective effect of these agents might be blunted as they are not taken on a daily basis in the same way oral contraceptives are. However, the studies led by Cramer and Rosenberg have suggested a reduction in risk of development of ovarian cancer associated with aspirin use (10,11).

Thus, the rational was established to study the ability of a COX-2 inhibitor to reduce the incidence of spontaneous development of genital tract adenocarcinoma in the laying hen, as this represents an animal model of ovarian carcinoma. Following a dose finding study, a controlled trial was completed, whereby, a COX-2 inhibitor was administered and hens exposed to this intervention for 12-22 months were assessed for the development of carcinoma. No significant difference was observed in our study suggesting the ability of a COX-2 inhibitor to prevent the development of genital tract adenocarcinoma in this animal model of ovarian cancer. One can speculate that an

inadequate dose was utilized or an inadequate length of exposure was utilized leading to the current results.

Alternatively, it is of significant interest that the COX-2 inhibitor intervention had no significant effect on ovulatory activity in the laying hen. However, the strongest support for the potential prevention of ovarian carcinoma lies in epidemiological studies of oral contraceptives. Multiple studies have demonstrated a risk reduction for the subsequent development of ovarian carcinoma of approximately 50% in regular users of oral contraceptives (19-36). These data strongly support a protective role of oral contraceptives against the development of ovarian carcinoma. Historically, the effect has been attributed to reduction in the number of ovulatory events associated with regular use of OCs. This theory had been supported by prior studies of progesterone derivatives in the laying hen model reported previously (37). In this study, a reduction in risk of development of genital tract adenocarcinoma was observed in those hens exposed to medroxyprogesterone which was noted to result in a dramatic but temporary reduction in ovulatory activity. When this study and the current results are taken in aggregate, there are further potential clues regarding the importance of ovulatory activity and the development of ovarian cancer that demand further investigation.

To date, chemoprevention of ovarian carcinoma represents an extremely neglected field of study. However, continued developments in molecular biology, biologic therapeutics, and pathogenesis/carcinogenesis are creating a solid rationale to explore chemoprevention as an approach to reduce deaths attributable to ovarian carcinoma. The ability of a chemopreventive agent to reduce the frequency of spontaneously arising reproductive tract adenocarcinomas in the avian hen model should stimulate further exploration of novel compounds in this context. Moreover, the

ability to perform further investigation into the molecular events associated with the generation of these cancers in the avian model may yield clues to the pathophysiology of human ovarian carcinoma.

REFERENCES

1. Partridge E, Barnes M. Epithelial ovarian cancer: Prevention, diagnosis, and treatment. *CA – A Cancer J Clinicians* 1999;49:297-316.
2. Grimes D. Primary prevention of ovarian cancer. *JAMA* 1993;270:2855-6.
3. Ahnen DJ. Colon cancer prevention by NSAIDs: What is the mechanism of action? *Eur J Surg Suppl.* 582:111-114, 1998.
4. Singh-Ranger G, Mokbel K. The role of cyclooxygenase-2 (COX-2) in breast cancer, and implications of COX-2 inhibition. *Eur J of Surgical Oncology.* 28; 729-737, 2002.
5. Rioux N, Castonguay A. Prevention of NK induced lung tumorigenesis in A/J mice by acetylsalicylic and NS398. *Cancer Res* 58; 5354-5360, 1998.
6. Ogino M, Minoura S. Indomethacin increases the cytotoxicity of cis-platinum and 5-fluorouracil in the human cervical cancer cell lines SKG-2 and HKUS by increasing the intracellular uptake of the agents. *Int J of Clinical Oncology* 6; 84-89, 2001.
7. Sawakoa H, Kawano S, Tsujii M, Murata H, Hori M. Effects of NSAIDs on proliferation of gastric cancer in vitro: possible implication of cyclooxygenase-2 in cancer development. *J Clinical Gastroenterolgy.* 27 suppl.; s47-s52, 1998.
8. Moysich KB, Mettlin C, Piver MS, et al. Regular use of analgesic drugs and ovarian cancer risk. [Cancer Epidemiol Biomarkers Prev](#) 10:903-906, 2001.

9. Tavani A, Gallus S, La Vecchia C, et al. Aspirin and ovarian cancer: An Italian case-control study. [Ann Oncol](#) 11:1171-1173, 2000.
10. Rosenberg L, Palmer JR, Rao RS, et al. A case-control study of analgesic use and ovarian cancer. [Cancer Epidemiol Biomarkers Prev](#) 9:933-937, 2000.
11. Cramer DW, Harlow BL, Titus-Ernstoff L, et al. Over-the-counter analgesics and risk of ovarian cancer. *Lancet* 351:104-107, 1998.
12. Fredrickson T. Ovarian tumors of the hen. *Env Health Perspectives* 1987;73: 35-51.
13. Papsolomontos P, Appleby E, Mayor O. Pathologic findings in condemned chickens: A survey of 1000 carcasses. *The veterinary record* 1969;85: 459-64.
14. Burford C, Barnes M, Berry W, Partridge E, Grizzle W. Immunohistochemical expression of molecular markers in an avian model: A potential model for preclinical avaluation of agents for ovarian cancer chemoprevention. *Gynecol Oncol* 2001;81: 373-379.
15. Alfonzso M, Adochiles L, Hendrickson V, Carver D, Rodriguez G, Barnes H. Metastatic adenocarcinoma in the lungs of older laying hens. *Avian Dis* 2005, 49: 430-2.
16. NIH Consensus Development Panel on Ovarian Cancer. Ovarian cancer; screening, treatment and follow-up. *JAMA* 1995;273:491-97.
17. Lundholm K, Gelin J, Hyltander A, Lonnroth C, Sandstrom R, Svaninger G, Korner U, Gulich M, Karrefors I, Norli B. Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumors. *Cancer Res* 54; 5602-5606, 1994

18. Rodriguez-Burford C, Barnes MN, Oelschlager DK, et al. Effects of non-steroidal anti-inflammatory agents (NSAIDs) on ovarian carcinoma cell lines: Preclinical evaluation of NSAIDs as chemopreventive agents. Clin Cancer Res 8:202-209, 2002.
19. Ness R, Grisso J, Vergona R, et al. Oral contraceptives, other methods of contraception, and risk reduction for ovarian cancer. Epidemiology 2001;12:307-312.
20. Siskind V, Green A, Bain C, et al. Beyond oral contraceptives and epithelial ovarian cancer. Epidemiology 2000;11:106-110.
21. Narod S, Risch H, Moslehi R, et al. Oral contraceptives and the risk of hereditary ovarian cancer. Hereditary ovarian cancer clinical study group. NEJM 1998;339:424-428.
22. Vessey M, Painter R. Endometrial and ovarian cancer and oral contraceptives- findings in a large cohort study. British J Cancer 1995;71:1340-1342.
23. Hankinson S, Colditz G, Hunter D, et al. A prospective study of reproductive factors and risk of epithelial ovarian cancer. Cancer 1995;76:284-289.
24. Rosenberg L, Palmer J, Zauber A, et al. A case control study of oral contraceptive use and invasive epithelial ovarian cancer. Am J Epidemiology 1994;139:654-661.
25. John E, Whittemore A, Harris R, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of seven US case control studies. Epithelial ovarian cancer in black women. JNCI 1993;85:142-147.
26. Parazzini F, La Vecchia C, Negri E, et al. Oral contraceptive use and the risk of ovarian cancer: an Italian case control study. European J Cancer 1991;27:594-598.

27. Franceschi S, Parazzini F, Negri, et al. Pooled analysis of 3 European case control studies of epithelial ovarian cancer. Oral contraceptive use. *Int J Cancer* 1991;49:61-65.
28. Parazzini F, Restelli C, La Vecchia C, et al. Risk factors for epithelial ovarian tumors of borderline malignancy. *Int J Epidemiology* 1991;20:871-877.
29. Gwinn M, Lee N, Rhodes P. Pregnancy, breast feeding, and oral contraceptives and the risk of ovarian cancer. *J Clinical Epidemiology* 1990;43:559-568.
30. Cancer and Steroid Hormone (CASH) Group. The reduction of risk of ovarian cancer associated with oral contraceptive use. *NEJM* 1987;316:650-655.
31. Tzonou A, Day N, Trichopoulos D, et al. The epidemiology of ovarian cancer in Greece: a case control study. *European J Cancer and Clin Oncol* 1984;20:1045-1052.
32. La Vecchia C, Franceschi S, Decarli A. Oral contraceptive use and the risk of epithelial ovarian cancer. 1984;50:31-34.
33. Rosenberg L, Shapiro S, Slone S, et al. Epithelial ovarian cancer and combination oral contraceptives. *JAMA* 1982;247:3210-3212.
34. Cramer D, Hutchinson G, Welch W. Factors affecting the association of oral contraceptives and ovarian cancer. *NEJM* 1982;307:1047-1051.
35. Willett W, Bain C, Hennekens C. Oral contraceptives and risk of ovarian cancer. *Cancer* 1981;48:1684-1687.
36. Weiss N, Lyon J, Liff J, et al. Incidence of ovarian cancer in relation to the use of oral contraceptives. *Int J Cancer* 1981;28:669-671.
37. Barnes M, Berry W, Straughn M, Kirby T, Leath C, Huh W, Grizzle W, Partridge E. A controlled trial of ovarian cancer chemoprevention using medroxyprogesterone

acetate in an avian model of spontaneous ovarian carcinogenesis. Gynecol Oncol
87, 57-63, 2002

APPENDICES

Appendix 1: Draft of Manuscript (see attached)

SUPPORTING DATA

Please reference tables included in body.

**CHEMOPREVENTION USING A COX-2 INHIBITOR IN AN AVIAN MODEL OF
SPONTANEOUS OVARIAN CARCINOGENESIS: CLUES TO THE IMPORTANCE OF
OVULATORY ACTIVITY IN THE PATHOGENESIS OF OVARIAN CANCER**

Mack N. Barnes MD, Wallace D. Berry PhD*, J. William E. Grizzle MD PhD**

Department of Gynecologic Oncology, **Department of Pathology, University of
Alabama at Birmingham, Birmingham AL, * School of Poultry Science, Auburn
University Auburn AL.

Support:

Department of Defense Grant (W81XWH-04-1-0322)

Correspondence: Mack N. Barnes, MD

Division of Gynecologic Oncology

University of Alabama at Birmingham

538 Old Hillman Bldg

618 South 20th St

Birmingham, AL 35233

(205) 934-4986

V6.19.02

Abstract

Objective: Due to epidemiological data suggesting a protective effect from non-steroidal anti-inflammatory drugs (NSAID), a rational exists for the utilization of these agents in the chemoprevention of ovarian carcinoma. Therefore, the objective of this study was to determine, in a controlled chemoprevention trial, the ability of a COX-2 inhibitor to inhibit the development of spontaneously arising genital tract adenocarcinoma in the laying hen (*Gallus Domesticus*) animal model of ovarian cancer.

Method: Following a dose finding study for the COX-2 inhibitor, Rimadyl, 480 hens (240 hens in treatment group / 240 hens in control group) were utilized in a controlled trial of this agent to determine the subsequent development of genital tract adenocarcinoma. A dose of 10mg/kg body weight of Rimadyl was utilized. The duration of treatment extended from 11/04 to 9/06. Following the duration of treatment, tissues from informative hens were examined histologically and the frequency of reproductive tract adenocarcinoma determined. **Results:** For analysis of “informative subjects,” 156 hens constituted the control group and 148 hens constituted the treatment group. Assessment of egg count data suggests no reduction in the number of eggs produced in the animals treated exposed to COX-2 inhibitor when compared to control. The overall rate of spontaneously arising reproductive tract adenocarcinomas was 64%. There was no evidence of a reduction of reproductive tract adenocarcinoma (Treatment group-64% vs Control Group-62%). **Conclusion:** In this study, no reduction in the incidence of genital tract adenocarcinoma was observed in the laying hen model following exposure to a COX-2 inhibitor. It is of significant interest that no effect on ovulatory activity was observed. This is particularly relevant given epidemiologic studies that link a reduction

of ovulatory activity to reduction of risk in ovarian cancer. This study potentially helps shed light on the importance of reducing ovulatory activity in the pursuit of ideal chemopreventive agents for ovarian cancer.

INTRODUCTION

Ovarian carcinoma is the fourth leading cause of cancer death in the female population and the most fatal gynecologic malignancy (1). Approximately, 75% of the cases of ovarian cancer present in an advanced stage where therapy consists of surgical debulking and adjuvant chemotherapy (1). Like many other disseminated solid tumors, studies to date that center on therapeutic strategies suggest it is unlikely that current treatment of advanced ovarian carcinoma will yield significant improvements in long term mortality from this insidious disease.

Chemoprevention strategies may provide a rational alternative approach for meaningful reductions in deaths attributable to ovarian carcinoma (2). Cancer chemoprevention refers to the prevention of cancer with drugs or natural substances which do not cause significant side effects when used chronically. Chemoprevention strategies may provide a rational alternative approach for meaningful reductions in deaths attributable to ovarian carcinoma (2). Non-steroidal anti-inflammatory drugs (NSAIDs) have generated significant enthusiasm as chemoprevention agents, particularly in the area of colon carcinoma (3). Moreover, preliminary data exists to suggest that these agents may also exhibit preventive effects in cancers of the breast, lung, cervix, and other components of the gastrointestinal tract (4-7). 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 (8-11). These studies are difficult to interpret due to the retrospective nature of the study design, the potential for recall bias, and the possibility that the protective effect of these agents might be blunted as they are not taken on a daily basis in the same way oral contraceptives are. However, a reduction in the risk of ovarian cancer of approximately 50% has been observed in some of these studies (10,11).

While a strong rationale for chemoprevention of ovarian carcinoma exists, a mechanism for the comprehensive evaluation of novel compounds is impeded by the lack of a validated animal model of induced or spontaneous ovarian carcinogenesis. Identification of spontaneous reproductive tract adenocarcinoma in the laying hen (*Gallus Domesticus*) may provide one answer to this dilemma (12-14). An evaluation of 200 two-year old hens documented a 4% rate of grossly identifiable reproductive tract adenocarcinomas with a histologic appearance similar to human epithelial ovarian cancers (14). Alfonso et al, demonstrated a 45% rate of ovarian and oviductal adenocarcinoma in 4 year old hens suggesting a higher incidence of cancer development as age increases (15).

Therefore, the purpose of this study was to explore the hypothesis that administration of the potential class of chemopreventive agents, COX-2 inhibitors, will result in a decreased rate of development of ovarian carcinoma in an avian model of spontaneous ovarian carcinogenesis. Two primary objectives were to be examined in the course of this study. The first objective was to determine a tolerable daily dose (mg/kg) of COX-2 inhibitor, admixed with standard feed, in the laying hen. The effect of varying doses of COX-2 inhibitor on egg-laying activity as a surrogate marker for

ovulatory frequency was assessed. The dose identified was then used in the controlled trial. The second primary objective was to determine, in a controlled chemoprevention trial, the ability of a COX-2 inhibitor to inhibit the development of spontaneously arising genital tract adenocarcinoma in the laying hen.

MATERIALS AND METHODS

COX-2 Inhibitor (Rimadyl) Dose Finding in the Avian Hen

Preliminary unpublished studies had suggested significant and lethal toxicity with the NSAID, indomethacin, when administered to laying hens (personal communication: W. Berry). In addition, there is a paucity of information regarding the use of COX-2 inhibitors in the avian hen. Rimadyl (Pharmacia Inc.) is a COX-2 specific inhibitor approved for use in veterinary medicine and available in a stable powder form. Once identified, the daily tolerated dose would then be used in the controlled trial.

Three dose levels were established as indicated in Table 1.

Rimadyl was added with other feed ingredients at the time feed was mixed in the feed mill and delivered to the hens. 5 hens were utilized per dose level for a total of 20 hens. The hens were monitored for egg laying activity as defined by percent egg laying activity control (0 mg/kg) and egg shell quality. In addition, toxicity was monitored based on general appearance, feather quality, evidence of gastrointestinal bleeding, and death. The planned dose for use in the study would be identified as the dose not associated with the aforementioned untoward toxicity.

Avian Study Population

Prior to initiation of the study, a power calculation was performed assuming a 40% incidence of genital tract adenocarcinoma. It was estimated that to identify a reduction in disease of 20% at the 0.05 level of significance a treatment group of 88 hens and a control group of 88 hens would be required. The informative cases were defined as those hens completing at least six months of a course of treatment. Hens that were living at the completion of the study period were sacrificed and included in the “informative group”. Significant attrition from non-malignant causes is known to occur as hens age, particularly past two years. Therefore, to initiate this study 480 hens (*Gallus Domesticus*) were acquired 8/3/04 (kindly provided by D Roland PhD, Professor of Nutrition, Auburn University). The breed of hens was designated as *Gallus Domesticus* and were two years of age upon study initiation.

In order to account for natural attrition, we established a treatment group (240 hens) and a control group (240 hens). It is our practice to allow a period of time for hens to “acclimate” and, therefore, a period of 8 weeks was utilized to allow the hens to acclimate. The controlled study was then initiated November 19, 2004, using a daily dose of Rimadyl of 10mg/kg body weight. A subjective increase in number of hens that were dying was recognized as they aged and, therefore, the remaining live hens were sacrificed and necropsy performed September 29, 2006. During the study period, animals were housed in animal care facilities provided by Auburn University School of Poultry Science under the direct supervision of one of the studies co-authors (W.D.B.). The chickens were maintained and sacrificed according to standards governing animal

investigations and provided by Institutional Animal Care and Use Committee at Auburn University. After the study period had elapsed, the animals were euthanized in accordance with the rules established by the Panel on Euthanasia of the American Veterinary Medical Association listed in the 1993 Report of the American Veterinary Medical Association Panel on Euthanasia. Additionally, in January of each year an “induced molt” was performed. This procedure entails limiting food and light until a 25% reduction in body weight from baseline is achieved in the hen. Induced molts restore normal egg laying activity in this species of captive fowl and is performed as part of the routine care of these animals irrespective of the ongoing study. If not performed, the egg laying/ovulatory activity of the control group would cease. In order to maintain a “standardized environment” both control and treated hens were subjected to annual induced molts.

At the time of necropsy, the presence or absence of ascites and carcinomatosis was noted. Additionally, at the time of necropsy, samples of ovary/oviduct were obtained if no gross evidence of cancer was present. If gross evidence of cancer was identified, a sample of the metastatic tumor as well as the ovary/oviduct were obtained. Only those cases where cancer involved the ovary/oviduct structure primarily (with or without the presence of metastatic disease) are reported as index cases of reproductive tract adenocarcinoma.

Monitoring of Egg-laying activity

Periodic monitoring of egg-laying activity in the respective study groups was performed. To assess egg laying activity, a cohort of control hens and “treated” hens were monitored on a weekly basis throughout the 24 month study period and egg production totaled. More specifically, egg laying activity was monitored in available hens from week 1 to week 97. The egg production rate of the two groups could then be compared to assess the effect of administration of COX-2 inhibitor on egg laying activity and an association with the development of genital tract adenocarcinoma.

Histologic Evaluation

Tissue specimens were fixed in neutral-buffered formalin and processed to paraffin blocks. The specimens were then taken to the UAB Ovarian Cancer Core pathology facility for further processing. The specimens were paraffin embedded and five-micron sections were cut from each paraffin block, stained with hematoxylin and eosin (H&E), and reviewed by a pathologist (W.E.G.) for presence or absence of reproductive tract adenocarcinoma. All specimens were reviewed by a single pathologist (W.E.G.) who had gained experience in the histopathology of avian reproductive tract adenocarcinomas during a previous study (14). As all cases had oviductal tissues available for histologic review, cases were subjectively divided into small, medium, and large volume of tumor for a more detailed analysis.

Statistical Analysis

A qualitative assessment of the influence of administration of the preventive agent, Rimadyl (COX-2 inhibitor) on egg laying activity was depicted graphically. The prevalence of reproductive tract adenocarcinoma was determined after histologic examination of material from the index case hens. The rate of cancer in the treatment group (148 informative cases) was then compared to the control group (156 informative cases). A risk ratio for the presence of reproductive tract adenocarcinoma and a 95% confidence interval calculated were to be calculated if a difference was observed.

Results

The initial dose finding study was completed at four dose levels of Rimadyl as follows; 0 mg/kg body weight (control), 0.25 mg/kg body weight, 5mg/kg body weight, and 10mg/kg body weight. Over an 8 week study period (8/04-10/04), no toxicity and no lethal events were noted. In addition, there did not appear to be any effect on ovulatory activity, even at the highest dose level. Egg laying activity was also monitored over the course of the 2 year study period. Given, the ability of these hens to tolerate the highest dose level, 10mg/kg body weight was determined to be the dose that would be utilized in the controlled trial.

A total of 480 laying hens were initially divided into a treatment and control group consisting of 240 hens each. Over a 2 year course of exposure to COX-2 inhibitor, 304 hens were subjected to necropsy and represent the “informative” index cases as these were felt to be adequately exposed to the intervention. For analysis of “informative subjects,” 156 hens constituted the control group and 148 hens constituted the treatment group. The 176 hens not included in the “informative cases” were those not

exposed to adequate COX-2 inhibition, hens that expired and were culled during “weekend cage maintenance”, and some that were not evaluated due to poor tissue depending on cause of death. These hens did not undergo histologic examination.

Assessment of egg count data suggests no reduction in the number of eggs produced in the animals treated exposed to COX-2 inhibitor when compared to control (Table 2). As noted previously, no difference in egg-laying activity was observed during the brief dose finding study. An overall decline in egg laying activity is observed in the entire cohort of animals as they aged into their fourth year of life, and this physiologic phenomenon has been observed in prior studies and does not appear to be influenced by administration of a COX-2 inhibitor. A notable decrease in egg production is observed during week 37-39, which is due to the aforementioned induced molt.

Tissue specimens from all 304 of the index (or informative) cases were examined histologically for the presence or absence of reproductive tract adenocarcinoma. Consistent with a previous report, multiple cases of histologically confirmed spontaneously arising reproductive tract adenocarcinomas were identified (12-15).

The overall rate of spontaneously arising reproductive tract adenocarcinomas were observed in the study cohort of 304 four year-old hens was 64% (genital tract adenocarcinoma observed in 194 out of 304 hens). Of the tissues examined from the informative cases, there was no evidence of a reduction of reproductive tract adenocarcinoma. In aggregate, 98 of 156 (62%) hens in the control group were noted to have histologic evidence of genital tract adenocarcinoma while 96 of 148 (64%) in the Rimadyl group had histologic evidence of genital tract adenocarcinoma. These results were not significantly different. A subgroup analysis was then performed to see if any

differences were present in volume of tumor present, and as table 3 indicates no significant differences were observed.

Discussion

The ability of chemopreventive strategies to impact the incidence and subsequent mortality rate of ovarian carcinoma has been a neglected field of study. However, renewed interest in prevention of ovarian cancer is surfacing given the minimal impact on long term survival of therapeutic strategies for advanced disease. Screening strategies for ovarian cancer also have been tested but, at present, there is little evidence for the success of this strategy given the prohibitively low prevalence of disease (16). Therefore, prevention of ovarian cancer may represent the most rational investigational strategy to obtain a reduction in death from disease.

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 (3). Moreover, preliminary data exists to suggest that these agents may also exhibit preventive effects in cancers of the breast, lung, cervix, and other components of the gastrointestinal tract (4-7). 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.

Intriguing studies have demonstrated an anti-proliferative effect of NSAIDs in ovarian cancer and other solid tumors. Indeed, a randomized trial comparing indomethacin to placebo in end stage patients with solid tumors revealed a surprising survival advantage of 510 days compared to 250 days ($p < 0.05$) in patients with metastatic cancer (17). Recent reports have also documented the ability of NSAIDs, and COX-2 inhibitors specifically, to inhibit ovarian tumor cell growth and result in apoptosis in primary ovarian cancer cell cultures (18). While these studies are provocative, they more strongly address the role of these agents as anti-neoplastic drugs rather than agents that either prevent the development of cancer or cause reversion of the malignant phenotype.

To more directly address the potential preventive role of NSAIDs, case-control studies of ovarian carcinoma have suggested a risk reduction with the use of some NSAID derivatives. (8-11). These studies are difficult to interpret due to the retrospective nature of the study design the potential for recall bias, and the possibility that the protective effect of these agents might be blunted as they are not taken on a daily basis in the same way oral contraceptives are. However, the studies led by Cramer and Rosenberg have suggested a reduction in risk of development of ovarian cancer associated with aspirin use (10,11).

Thus, the rational was established to study the ability of a COX-2 inhibitor to reduce the incidence of spontaneous development of genital tract adenocarcinoma in

the laying hen, as this represents an animal model of ovarian carcinoma. Following a dose finding study, a controlled trial was completed, whereby, a COX-2 inhibitor was administered and hens exposed to this intervention for 12-22 months were assessed for the development of carcinoma. No significant difference was observed in our study suggesting the ability of a COX-2 inhibitor to prevent the development of genital tract adenocarcinoma in this animal model of ovarian cancer. One can speculate that an inadequate dose was utilized or an inadequate length of exposure was utilized leading to the current results.

Alternatively, it is of significant interest that the COX-2 inhibitor intervention had no significant effect on ovulatory activity in the laying hen. However, the strongest support for the potential prevention of ovarian carcinoma lies in epidemiological studies of oral contraceptives. Multiple studies have demonstrated a risk reduction for the subsequent development of ovarian carcinoma of approximately 50% in regular users of oral contraceptives (19-36). These data strongly support a protective role of oral contraceptives against the development of ovarian carcinoma. Historically, the effect has been attributed to reduction in the number of ovulatory events associated with regular use of OCs. This theory had been supported by prior studies of progesterone derivatives in the laying hen model reported previously (37). In this study, a reduction in risk of development of genital tract adenocarcinoma was observed in those hens exposed to medroxyprogesterone which was noted to result in a dramatic but temporary reduction in ovulatory activity. When this study and the current results are taken in aggregate, there are further potential clues regarding the importance of ovulatory activity and the development of ovarian cancer that demand further investigation.

In this study, no reduction in the incidence of genital tract adenocarcinoma was observed in the laying hen model following exposure to a COX-2 inhibitor. It is of significant interest that no effect on ovulatory activity was observed. This is particularly relevant given epidemiologic studies that link a reduction of ovulatory activity to reduction of risk in ovarian cancer. This study potentially helps shed light on the importance of reducing ovulatory activity in the pursuit of ideal chemopreventive agents for ovarian cancer.

REFERENCES

1. Partridge E, Barnes M. Epithelial ovarian cancer: Prevention, diagnosis, and treatment. *CA – A Cancer J Clinicians* 1999;49:297-316.
2. Grimes D. Primary prevention of ovarian cancer. *JAMA* 1993;270:2855-6.
3. Ahnen DJ. Colon cancer prevention by NSAIDs: What is the mechanism of action? *Eur J Surg Suppl.* 582:111-114, 1998.
4. Singh-Ranger G, Mokbel K. The role of cyclooxygenase-2 (COX-2) in breast cancer, and implications of COX-2 inhibition. *Eur J of Surgical Oncology.* 28; 729-737, 2002.
5. Rioux N, Castonguay A. Prevention of NK induced lung tumorigenesis in A/J mice by acetylsalicylic and NS398. *Cancer Res* 58; 5354-5360, 1998.
6. Ogino M, Minoura S. Indomethacin increases the cytotoxicity of cis-platinum and 5-fluorouracil in the human cervical cancer cell lines SKG-2 and HKUS by increasing the intracellular uptake of the agents. *Int J of Clinical Oncology* 6; 84-89, 2001.

7. Sawakoa H, Kawano S, Tsujii M, Murata H, Hori M. Effects of NSAIDs on proliferation of gastric cancer in vitro: possible implication of cyclooxygenase-2 in cancer development. *J Clinical Gastroenterolgy*. 27 suppl.; s47-s52, 1998.
8. Moysich KB, Mettlin C, Piver MS, et al. Regular use of analgesic drugs and ovarian cancer risk. [*Cancer Epidemiol Biomarkers Prev* 10:903-906, 2001.](#)
9. Tavani A, Gallus S, La Vecchia C, et al. Aspirin and ovarian cancer: An Italian case-control study. [*Ann Oncol* 11:1171-1173, 2000.](#)
10. Rosenberg L, Palmer JR, Rao RS, et al. A case-control study of analgesic use and ovarian cancer. [*Cancer Epidemiol Biomarkers Prev* 9:933-937, 2000.](#)
11. Cramer DW, Harlow BL, Titus-Ernstoff L, et al. Over-the-counter analgesics and risk of ovarian cancer. *Lancet* 351:104-107, 1998.
12. Fredrickson T. Ovarian tumors of the hen. *Env Health Perspectives* 1987;73: 35-51.
13. Papsolomontos P, Appleby E, Mayor O. Pathologic findings in condemned chickens: A survey of 1000 carcasses. *The veterinary record* 1969;85: 459-64.
14. Burford C, Barnes M, Berry W, Partridge E, Grizzle W. Immunohistochemical expression of molecular markers in an avian model: A potential model for preclinical avaluation of agents for ovarian cancer chemoprevention. *Gynecol Oncol* 2001;81: 373-379.
15. Alfonzso M, Adochiles L, Hendrickson V, Carver D, Rodriguez G, Barnes H. Metastatic adenocarcinoma in the lungs of older laying hens. *Avian Dis* 2005, 49: 430-2.

16. NIH Consensus Development Panel on Ovarian Cancer. Ovarian cancer; screening, treatment and follow-up. JAMA 1995;273:491-97.
17. Lundholm K, Gelin J, Hyltander A, Lonnroth C, Sandstrom R, Svaninger G, Korner U, Gulich M, Karrefors I, Norli B. Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumors. Cancer Res 54; 5602-5606, 1994
18. Rodriguez-Burford C, Barnes MN, Oelschlager DK, et al. Effects of non-steroidal anti-inflammatory agents (NSAIDS) on ovarian carcinoma cell lines: Preclinical evaluation of NSIADs as chemopreventive agents. Clin Cancer Res 8:202-209, 2002.
19. Ness R, Grisso J, Vergona R, et al. Oral contraceptives, other methods of contraception, and risk reduction for ovarian cancer. Epidemiology 2001;12:307-312.
20. Siskind V, Green A, Bain C, et al. Beyond oral contraceptives and epithelial ovarian cancer. Epidemiology 2000;11:106-110.
21. Narod S, Risch H, Moslehi R, et al. Oral contraceptives and the risk of hereditary ovarian cancer. Hereditary ovarian cancer clinical study group. NEJM 1998;339:424-428.
22. Vessey M, Painter R. Endometrial and ovarian cancer and oral contraceptives- findings in a large cohort study. British J Cancer 1995;71:1340-1342.
23. Hankinson S, Colditz G, Hunter D, et al. A prospective study of reproductive factors and risk of epithelial ovarian cancer. Cancer 1995;76:284-289.

24. Rosenberg L, Palmer J, Zauber A, et al. A case control study of oral contraceptive use and invasive epithelial ovarian cancer. *Am J Epidemiology* 1994;139:654-661.
25. John E, Whittemore A, Harris R, et al. Characteristics relating to ovarian cancer risk: collaborative analysis of seven US case control studies. Epithelial ovarian cancer in black women. *JNCI* 1993;85:142-147.
26. Parazzini F, La Vecchia C, Negri E, et al. Oral contraceptive use and the risk of ovarian cancer: an Italian case control study. *European J Cancer* 1991;27:594-598.
27. Franceschi S, Parazzini F, Negri, et al. Pooled analysis of 3 European case control studies of epithelial ovarian cancer. Oral contraceptive use. *Int J Cancer* 1991;49:61-65.
28. Parazzini F, Restelli C, La Vecchia C, et al. Risk factors for epithelial ovarian tumors of borderline malignancy. *Int J Epidemiology* 1991;20:871-877.
29. Gwinn M, Lee N, Rhodes P. Pregnancy, breast feeding, and oral contraceptives and the risk of ovarian cancer. *J Clinical Epidemiology* 1990;43:559-568.
30. Cancer and Steroid Hormone (CASH) Group. The reduction of risk of ovarian cancer associated with oral contraceptive use. *NEJM* 1987;316:650-655.
31. Tzonou A, Day N, Trichopoulos D, et al. The epidemiology of ovarian cancer in Greece: a case control study. *European J Cancer and Clin Oncol* 1984;20:1045-1052.
32. La Vecchia C, Franceschi S, Decarli A. Oral contraceptive use and the risk of epithelial ovarian cancer. 1984;50:31-34.

33. Rosenberg L, Shapiro S, Slone S, et al. Epithelial ovarian cancer and combination oral contraceptives. JAMA 1982;247:3210-3212.
34. Cramer D, Hutchinson G, Welch W. Factors affecting the association of oral contraceptives and ovarian cancer. NEJM 1982;307:1047-1051.
35. Willett W, Bain C, Hennekens C. Oral contraceptives and risk of ovarian cancer. Cancer 1981;48:1684-1687.
36. Weiss N, Lyon J, Liff J, et al. Incidence of ovarian cancer in relation to the use of oral contraceptives. Int J Cancer 1981;28:669-671.
37. Barnes M, Berry W, Straughn M, Kirby T, Leath C, Huh W, Grizzle W, Partridge E. A controlled trial of ovarian cancer chemoprevention using medroxyprogesterone acetate in an avian model of spontaneous ovarian carcinogenesis. Gynecol Oncol 87, 57-63, 2002

Table 1. Dose levels utilized during initial dose finding study for Rimadyl.

<u>Cohort</u>	<u>Dose of COX-2 Inhibitor</u>
---------------	--------------------------------

Control: 0 mg/kg body weight

Dose 1: 2.5 mg/kg body weight

Dose 2: 5 mg/kg body weight

Dose 3: 10 mg/kg body weight

Table 2. Egg Laying Production of COX-2 Exposed Hens Compared To Control Hens During Week 1 Through 97 Of The Study Period.

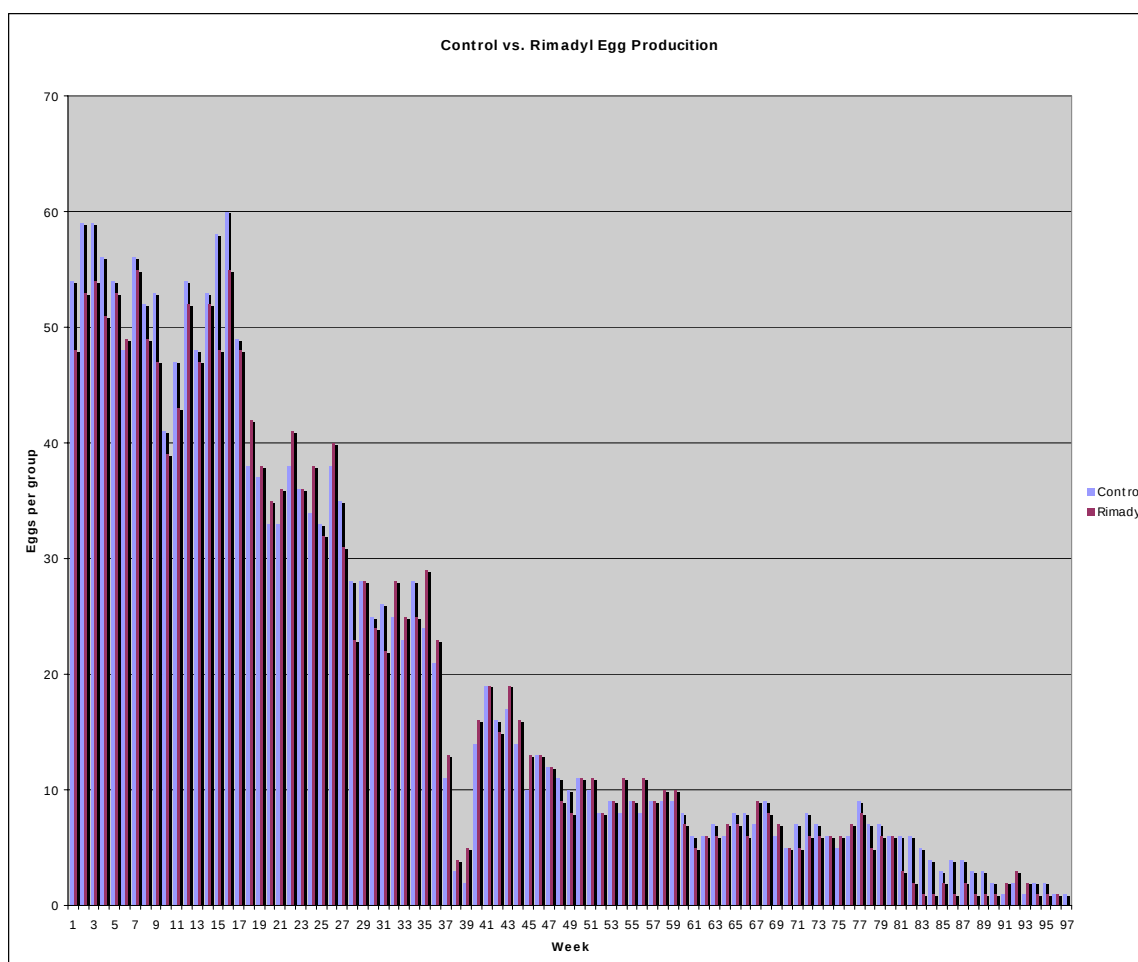


Table 3. Evaluation Of Volume Of Tumor Assessed In Hens Exposed to Rimadyl Versus Control.

<u>Volume of Tumor</u>	<u>Control</u>	<u>Rimadyl</u>	<u>Difference</u>
No Tumor	58	52	n.s.
Small	13	17	n.s.
Moderate	23	15	n.s.
Large	62	64	n.s.