

AD _____

Award Number: DAMD17-00-1-0118

TITLE: Genistein Programming Against Breast Cancer

PRINCIPAL INVESTIGATOR: Coral A. Lamartiniere, Ph.D.

CONTRACTING ORGANIZATION: The University of Alabama at Birmingham
Birmingham, Alabama 35294-0111

REPORT DATE: September 2001

TYPE OF REPORT: Annual

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.

20020118 150

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

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 Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 2001	3. REPORT TYPE AND DATES COVERED Annual (01 Sep 00 - 31 Aug 01)	
4. TITLE AND SUBTITLE Genistein Programming Against Breast Cancer			5. FUNDING NUMBERS DAMD17-00-1-0118	
6. AUTHOR(S) Coral A. Lamartiniere, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Alabama at Birmingham Birmingham, Alabama 35294-0111 E-Mail: Coral.Lamartiniere@ccc.uab.edu			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. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. Abstract (<i>Maximum 200 Words</i>) (<i>abstract should contain no proprietary or confidential information</i>) Most soy-breast cancer epidemiological studies conclude that Asian women consuming a traditional diet high in soy products have a low incidence of breast cancer. We have demonstrated that prepubertal exposure to genistein, the primary isoflavone of soy, protects against chemically-induced mammary cancer. The purpose of this work was/is to determine if adult exposure to genistein will protect against chemically-induced mammary cancer and to investigate DNA methylation of estrogen receptor genes as the molecular mechanism of genistein chemoprevention. To date, we have determined that adult only exposure to genistein does not protect against dimethylbenz(a)anthracene-induced mammary cancer. However, prepubertal plus adult exposure to 250 mg genistein/kg AIN-76A diet protected against DMBA-induced mammary cancer. This suggest that exposure to genistein prepubertally may imprint molecular events in the mammary gland that determines the "blue print" from which the mammary cells responds to future hormonal and/or xenobiotic response. In the second year, we will investigate DNA methylation of estrogen receptor genes as the molecular mechanism for genistein imprinting against mammary cancer.				
14. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award) Genistein, Rat, Breast, Cancer Chemoprevention			15. NUMBER OF PAGES 8	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	5
Appendices.....	6

INTRODUCTION

We have previously demonstrated that short-term exposure of rats to genistein, a soy phytoestrogen, early in postnatal life suppressed chemically-induced mammary cancer in adulthood (1, 2). This novel finding supports the epidemiological reports that Asian women consuming a traditional diet high in soy products have a low incidence of breast cancer (3-5). Furthermore, adjustment for migration rates of Asians to the U.S. revealed that the second, but not the first, generation lose this protection (6). This suggests that exposure to soy early in life confers life-time protection against breast cancer. Since short-term genistein treatment early in postnatal life exerted long-term protection against chemically-induced mammary cancer, we have hypothesized that genistein caused this effect via an imprinting mechanism. For this research, we proposed to investigate 1) the potential of adult genistein treatment to alter susceptibility for breast cancer and 2) DNA methylation of estrogen receptor genes as the molecular mechanism of action.

BODY

Specific Aim 1) To determine the risk of mammary cancer from adult exposure to genistein. This was investigated in the rat-DMBA mammary cancer model. (To be carried out in the first year.)

In the first experiment, we have investigated 2 groups of rats fed AIN-76A diet since parturition, and treated with DMBA on day 50. At day 100 postpartum (shortly after the first tumors can be palpated), we switched one group to 250 mg genistein/kg AIN-76A diet. The results showed no significant difference in tumor formation or adenocarcinoma development (Figure 1). We concluded that 1) rats not exposed to genistein prepubertally do not receive protection from genistein after tumors have developed, 2) genistein does not promote existing mammary tumors, and 3) that genistein exposure must occur prepubertally to exert a chemopreventive effect.

In the second tumorigenesis experiment, we investigated the potential of a combination of prepubertal and adult genistein exposure to protect against DMBA-induced mammary cancer. The purpose of this experiment was to determine if early critical exposure (prepubertal) to genistein would influence how the adult animal would respond to future genistein treatment. Group 1 was fed AIN-76A diet containing 250 mg genistein/kg diet, starting from parturition through day 21 only, and then AIN-76A onward (Gen/DMBA/Zero). Group 2 was fed the genistein diet from parturition through day 21, then AIN-76A only through day 100 postpartum and then from day 100, the genistein-containing diet (Gen/DMBA/Gen). All animals received 80 mg DMBA/kg BW at day 50. As seen in Figure 2, genistein fed to adults already exposed to genistein prepubertally (Gen/DMBA/Gen) had an added level of protection.

Specific Aim 2) To investigate genistein imprinting by methylation of estrogen receptor genes as the mechanism for mammary cancer prevention. This is currently under investigation.

KEY RESEARCH ACCOMPLISHMENTS

- 1) Dietary genistein given to adult female rats after tumors were initiated, did not alter the multiplicity of mammary tumors. This can be interpreted as genistein not exerting a chemotherapeutic effect on existing tumors, and genistein not exacerbating development of previously existing mammary tumors.
- 2) On the other hand, dietary genistein to adult rats exposed prepubertally to genistein provides additional protection against mammary cancer. Prepubertal genistein exposure appears to "imprint for additional adult genistein chemoprevention.

REPORTABLE OUTCOMES

This data have been presented in part at the following meetings:

University of Missouri Sixth Annual Oncology Conference. Dietary Genistein Protects against Mammary and Prostate Cancers. Lake of the Ozarks, Missouri. April 27, 2001

FASEB Summer Research Conference on Physiological Functions of Antioxidant Nutrients and Phytochemicals. Genistein and Breast and Prostate Cancers. Tucson, AR. June 16-21, 2001

Hormonal Carcinogenesis Gordon Conference. Dietary Factors in Hormonal Carcinogenesis. Genistein Chemoprevention: Timing of Exposure and Mechanisms of Action (Mammary and Prostate). Kimball, NH, July 8-13, 2001.

CONCLUSIONS

We conclude that dietary genistein in adult life is only effective in protecting against chemically-induced mammary cancer if the female mammary gland has already been imprinted prepubertally.

REFERENCES

1. Fritz, W.A., Coward, L., Wang, J. and Lamartiniere, C.A. Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. *Carcinogenesis* 19: 2151-2158, 1998.
2. Lamartiniere, C.A., Zhao, Y.-X. and Fritz, W.A. Genistein: mammary cancer chemoprevention, in vivo mechanisms of action, potential for toxicity, and bioavailability in rats. *Journal of Women's Cancer*, 2: 11-19, 2000.
3. Lee, H.P., Gourley, L., Duffy, S.W., Esteve, J., Lee, J. and Day, N.E. Dietary effects on breast cancer risk in Singapore. *Lancet*. 336: 1197-1200, 1991.
4. Yuan, J-M., Wang, Q-S., Ross, R.K., Henderson, B.E. and Yu, M.C. Diet and risk of breast cancer in Shanghai and Tianjin, China. *Brit. J. Cancer* 71: 1353, 1995.

5. Wu, A.H., Zeigler, R.G., Horn-Ross, P.L., Nomura, A.M., West, D.W., Kolonel, L.N., Rosenthal, J.F., Hoover, R.N. and Pike, M.C. Tofu and risk of breast cancer in Asian-Americans. *Cancer Epi. Bio. Prev.* 5: 901-906, 1996.
6. Ziegler, R.G., Hoover, R.N., Hildeshein, R.N., Nomura, M.Y., Pike, M.C., West, D.W., Wu-Williams, A., Kolonel, L.N., Horn-Ross, P.L., Rosenthal, J.F. and Hyer, M.B. Migration patterns and breast cancer risk in Asian-American women. *J. Natl. Cancer* 85: 1819, 1993.

APPENDICES

Two figures

Post DMBA Genistein Treatment

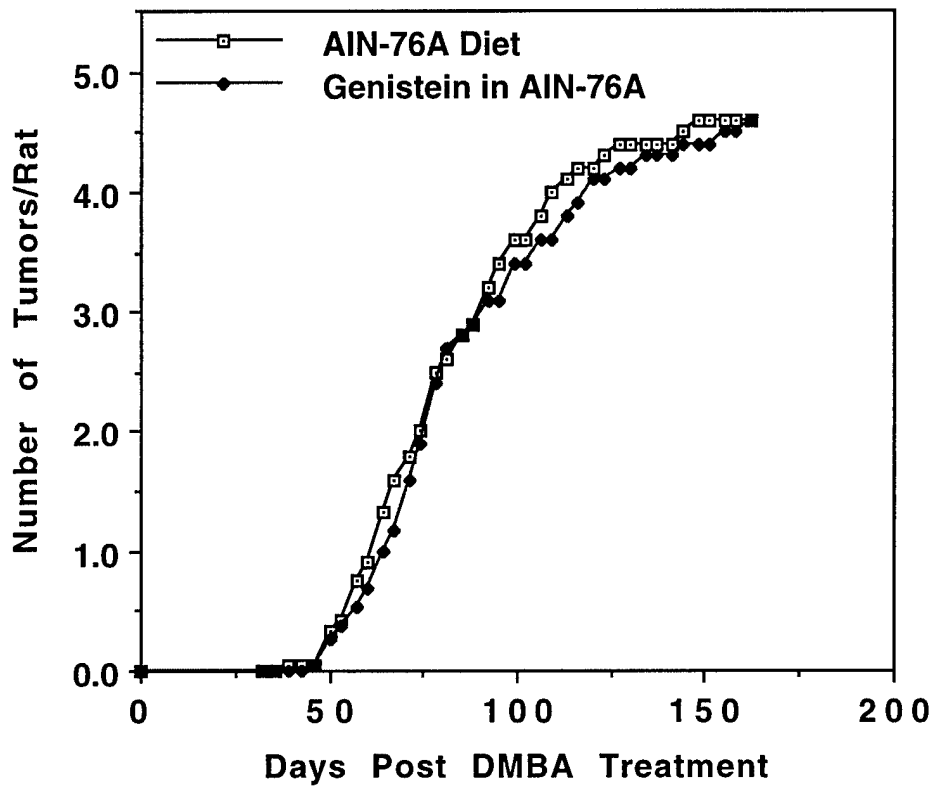


Figure 1. Adult dietary genistein effect on mammary tumors in rats exposed as adults to 80 mg DMBA/kg body weight at day 50 postpartum. One group was fed AIN-76A diet from parturition onward (AIN-76A Diet). The second group was fed AIN-76A diet from parturition through day 100 (50 days post-DMBA), then they were fed 250 mg genistein/kg AIN-76A diet (Genistein in AIN-76A).

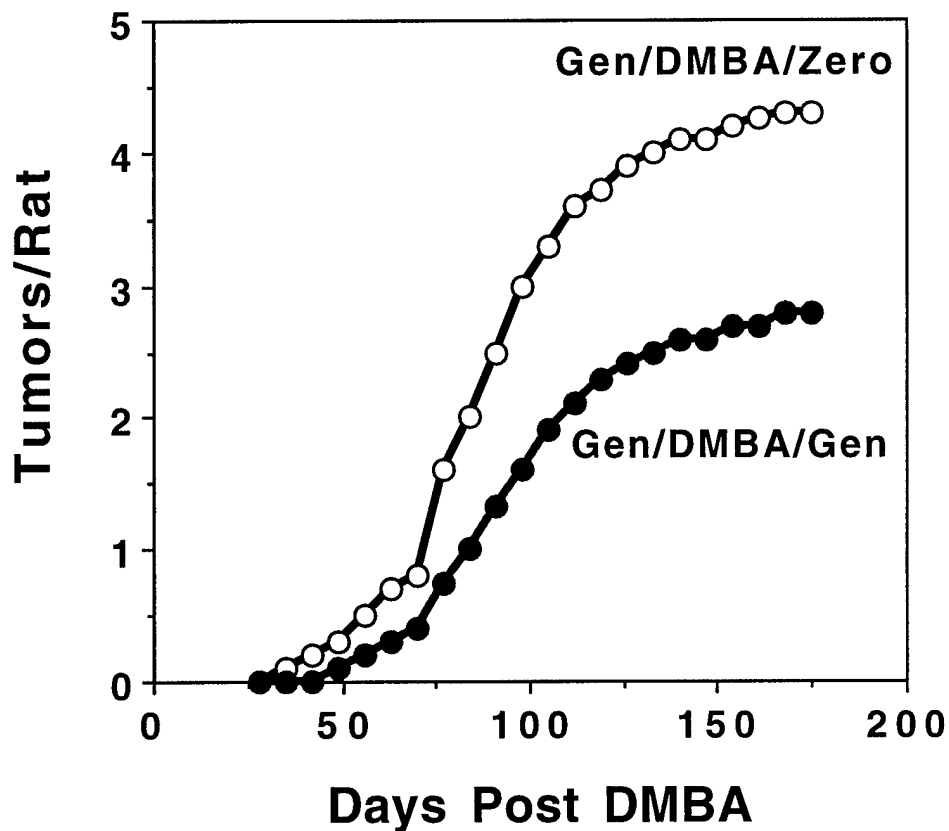


Figure 2. Adult dietary genistein effect on palpable mammary tumors in rats exposed prepubertally to genistein, and as adults to DMBA. One group was fed AIN-76A diet containing 250 mg genistein/kg diet, starting from parturition through day 21 only and then AIN-76A onward (Gen/DMBA/Zero). The second group was fed genistein containing diet from parturition through day 21, then AIN-76A only through day 100 postpartum and then from day 100, genistein containing diet (Gen/DMBA/Gen). All animals received 80 mg DMBA/kg body weight at day 50. Day 100 postpartum is 50 days after the DMBA was administered, shortly after the first tumors were palpable.