Neonatal Genistein Chemoprevents Mammary Cancer (43843)

CORAL A. LAMARTINIERE,[1] JUDSON MOORE, MICHAEL HOLLAND, AND STEPHEN BARNES

Departments of Pharmacology and Toxicology, and Environmental Health Sciences, and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, Alabama 35294-0019

Abstract. **We have investigated the potential of genistein, an estrogenic component of soy, when administered neonatally, to manifest a protective effect against chemically induced mammary cancer. Female Sprague-Dawley rats were treated on Day 2, 4, and 6 postpartum with genistein or dimethylsulfoxide (vehicle). To induce mammary carcinogenesis, all animals were subsequently exposed on Day 50 postpartum to dimethylbenz(a)anthracene. Animals treated neonatally with genistein had increased latency and reduced incidence and multiplicity of mammary tumors compared with vehicle-treated animals. Cell differentiation studies in mammary whole mounts revealed that neonatal genistein treatment resulted in decreased numbers of terminal end buds and increased numbers of lobular structures. A precocious maturation of undifferentiated terminal end buds to more differentiated lobules may account for neonatal genistein treatment protecting against chemically induced mammary cancer.** [P.S.E.B.M. 1995, Vol 208]

Breast cancer is the most common cancer among U.S. females and is the second leading cause of cancer death among women. In the last decade, the incidence of breast cancer has increased at a rate of approximately 2% per year (1). There is a concern that our industrialization has contributed to this increase via the production and contamination of our environment with estrogenically active xenobiotics (2). On the other hand, there may also be the potential for protection against hormone-responsive cancers from the phytochemicals in the food we eat.

Our investigations into nutritional prevention of breast cancer occurred as a result of the serendipitous observation that neonatal estrogen treatment inhibited the appearance of spontaneous and chemically induced mammary tumors (3, 4). Since such estrogens (diethylstilbestrol) are also associated with other types of cancer, we sought an estrogenically active component to prevent mammary cancer which has little or no toxic effects. Such a compound is the isoflavone genistein, a phytochemical component of soybeans.

Genistein is a diphenolic planar molecule with an aromatic A-ring. It has a second oxygen atom 11.5 Å from the one in the A-ring and a molecular weight similar to those of the steroidal estrogens. Genistein has been shown to have estrogenic properties in receptor binding assays (5, 6), cell cultures (7), and uterine weight assays (8–10). It has also been shown to inhibit DNA topoisomerase activity (11) and tyrosine protein kinases (12). While genistein given at high doses to neonatal rats has been reported to alter the hypothalamic-pituitary axis (13), we know of no reports that soy products cause any adverse developmental effects in humans. In the rat, Barnes and colleagues have shown that powdered soybean chips, isolated soy protein, and soy molasses can reduce the number of mammary tumors induced by dimethylbenz(a)anthracene (DMBA) and N-nitroso-N-methylurea (NMU) (14, 15). Other researchers have shown that isolated soy protein is chemopreventive in the NMU model of breast cancer, even when given five weeks after initiation by NMU, suggesting that it may have antipromoter effects (16).

Oriental women and men, consuming a traditional diet high in soy products, are reported to have low incidences of breast and prostate cancer (17, 18). Soy-

[1] To whom requests for reprints should be addressed at Department of Pharmacology and Toxicology, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294-0019.

0037-9727/95/2081–0000$10.50/0

based diets contain high levels of isoflavones, and isoflavonic phytoestrogens including genistein are normal constituents of human urine from subjects consuming large amounts of whole-grain products and vegetables high in soy products (18). It was the objective of this study to investigate the potential of genistein when given during the neonatal period to protect against chemically induced mammary cancer in the rat.

Materials and Methods

Sprague-Dawley-CD timed pregnant rats were obtained from Charles River Breeding Laboratories, Raleigh, NC. At the time of birth, all dams were transferred from Prolab 3000 animal diet (Agway Inc., Syracuse, NY) to AIN-76A diet (Madison, WI). AIN-76A is a semipurified diet that does not contain "estrogens." The offspring were sexed, all females retained and randomly distributed to the dams with enough males added to leave 10 offspring/litter. After ear marking the offspring, one-half of the females from each litter received 5 mg genistein in 20 μl dimethylsulfoxide (DMSO) and the rest of the females received the vehicle only. This treatment was administered sc on Day 2, 4, and 6 postpartum (day of birth counted as Day 1). Animals were weaned at Day 23 postpartum. At Day 50 postpartum all animals received, via gavage, 8 mg DMBA/100 g body weight. Animals were subsequently palpated weekly for mammary tumors until they reached the age of 230 days, the tumors exceeded 2.5 cm in diameter or the animals became moribund. The location and time of appearance of each tumor were charted. Upon necropsy, all tumors were excised, recorded as to location and weighed.

Mammary whole mounts of the superior abdominal glands were prepared by the methods of Banerjee *et al.* (19) and the Russos (20). The glands were excised, spread on slides and processed for staining with alum carmine, dehydrated in graded alcohols, cleared in xylene, and then mounted on a slide with Permount. Coded whole mounts were examined under light-microscopy at 40× and 100× and scored for the number and ratio of terminal end buds, terminal ducts, and lobules. The criteria for identification of the structures are based on the work of Russo *et al.* (21, 22).

The tumorigenesis data were analyzed using the mathematical modeling approach proposed by Kokoska *et al.* (23). Standard analyses were conducted using the Wilcoxon rank sum test and the Fisher exact test.

Results

The tumorigenesis data revealed significant differences in the distribution of tumor appearance times between the two groups (Fig. 1). The mean time to detection in animals treated neonatally with DMSO as compared with animals treated neonatally with genistein was 87 ± 37 and 124 ± 33 days, respectively ($P < 0.001$). At 190 days post-DMBA treatment, 100% of the DMSO-treated animals had tumors, while only 88% of the genistein-treated animals had tumors. The mean numbers ±SEM of tumors per animal were 6.35 ± 0.67 and 3.67 ± 0.45 for DMSO- and genistein-treated animals, respectively ($P \leq 0.001$). Similar body weights were recorded for animals treated neonatally with genistein or with the vehicle, DMSO.

We subsequently investigated the effects of neonatal genistein treatment on the developmental maturation of the mammary gland in animals not receiving the DMBA treatment. Whole mounts of the superior abdominal glands of 50- and 90-day-old female rats treated neonatally with genistein and DMSO were examined for their state of differentiation (8, 19). At 50 days of age, the mammary glands of genistein-treated females had significantly fewer terminal end buds and more lobules than glands from DMSO-treated females (Table I). At 90 days of age, there were very few terminal end buds and there were significantly greater numbers of lobules III in the genistein-treated animals ($P < 0.001$).

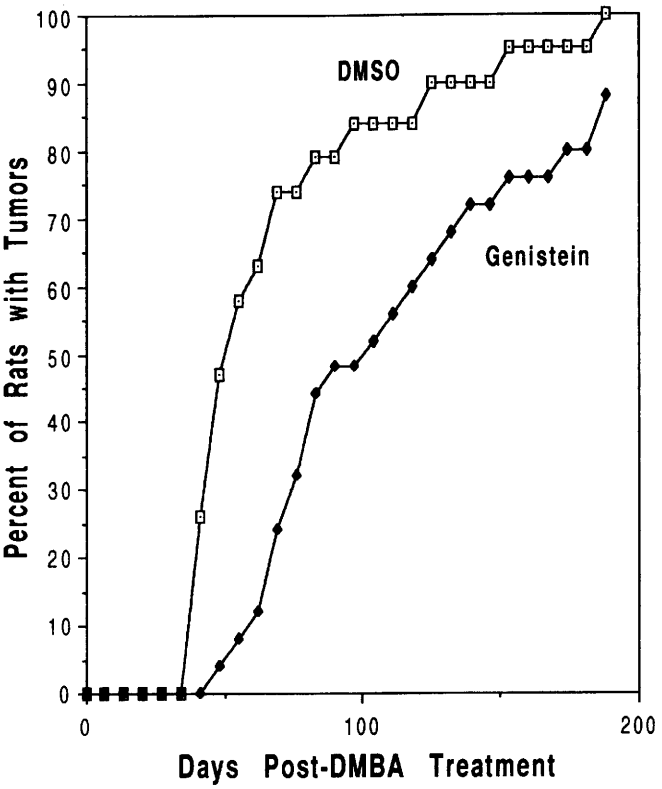

Figure 1. Ontogeny of palpable mammary tumors in female rats treated neonatally with genistein and dimethylsulfoxide (DMSO) and at Day 50 postpartum with dimethylbenz(a)anthracene (DMBA). Female Sprague-Dawley rats were treated (sc) with 5 mg genistein or 20 μl DMSO on Day 2, 4, and 6 postpartum. DMBA was administered by gavage at 8 mg/100 g body weight.

Table I. Effect of Neonatal Genistein Treatment on Percentage of Terminal Ductal Structures in the Mammary Glands of Fifty and Ninety Day Old Female Rats

Neonatal treatment	Terminal end buds	Terminal ducts	Lobules		
			I	II	III
In 50-day-old rats					
DMSO (14)	44 ± 3	30 ± 3	26 ± 5	0	0
Genistein (14)	34 ± 3[a]	31 ± 2	28 ± 3	6 ± 4	1 ± 1
In 90-day-old rats					
DMSO (6)	0	20 ± 4	20 ± 2	59 ± 4	1 ± 1
Genistein (5)	1 ± 1	13 ± 6	13 ± 5	37 ± 10	35 ± 19[b]

Note. Sprague-Dawley female rats were injected (sc) with 5 mg genistein or 20 μl DMSO on days 2, 4, and 6 postpartum. [a]$P < 0.05$; [b]$P < 0.001$ compared with neonatal DMSO treatment.

Discussion

We are the first to report that genistein, a phytoestrogen component of soy, when given during the neonatal period to rats results in protection against chemically induced mammary tumors. We have demonstrated that genistein as compared with DMSO administration on days 2, 4, and 6 postpartum resulted in a significantly longer period of time for DMBA-induced mammary tumors to develop. The latency was extended by 37 days ($P \leq 0.001$). At time of necropsy, 100% of the females treated neonatally with DMSO had tumors. Female rats treated neonatally with genistein had an 88% incidence of mammary tumors. The mean numbers of DMBA-induced tumors in the DMSO-treated group were 6.35 as compared with only 3.67 in animals treated with genistein ($P \leq 0.001$). There was no effect of the genistein treatment on the body weights of these animals.

An investigation into gland morphology revealed that the profile of ductal structures was different in animals treated neonatally with genistein as compared with animals treated neonatally with DMSO. At Day 50 postpartum (i.e. at the time that the carcinogen was administered in the tumorgenesis study), genistein-treated animals had significantly fewer terminal end buds, similar numbers of terminal ducts and lobules I, and increased numbers of lobules II. The decrease in terminal end buds and increase in lobules II suggest a more differentiated gland (21, 22). In 90-day-old females, we saw fewer terminal end buds and lobules I and II with a concomitant increase in lobules III. Lobules III are the most differentiated ductal structures in the mammary (21, 22).

A correlation between the status of mammary differentiation and protection against chemically induced mammary tumors can be made. According to the Russos (21, 22), terminal end buds and terminal ducts are undifferentiated ductal structures which are most susceptible to chemical carcinogens and can result in the formation of adenocarcinomas. Lobules I, II, and III are progressively more differentiated structures that are less susceptible to the formation of adenocarcinomas. Benign lesions usually arise from structures that are more differentiated at the time of carcinogen administration.

We surmise that genistein treatment during this early critical period of development has altered the ontogeny of mammary development, resulting in a more mature gland consisting of less susceptible structures. This may have occurred as a result of direct estrogenic action from genistein on the mammary cells to promote maturation, and/or from alterations of imprinting mechanisms (24) from exposure of the neonate to this phytoestrogen. The latter mechanism could be due to changing the regulation and expression of the hypothalamic-pituitary-gonadal axis and hence altering the development and susceptibility of the mammary ductal structures.

This research was supported by grants from the American Institute for Cancer Research (925G12), National Cancer Institute (CA13148S), and National Institute of Environmental Health Sciences (ES04360). We thank Mr. H. Banks and Ms. M. Thompson for technical assistance.

1. Cancer Facts and Figures—1993. Atlanta: American Cancer Society, p1, 1993.
2. Raloff J. EcoCancers: Do environmental factors underlie a breast cancer epidemic? Science News **144:**10–13, 1993.
3. Lamartiniere CA, Holland MB. Neonatal diethylstilbestrol prevents spontaneously developing mammary tumors. In: Li JJ, Nandi S, Li SA, Eds. Hormonal Carcinogenesis. Berlin: Springer Verlag, pp305–308, 1992.
4. Holland MB, Lamartiniere CA. Neonatal diethylstilbestrol chemoprevents mammary adenocarcinomas in rats. Proc Am Assoc Cancer Res **33:**167, 1992.
5. Shutt DA, Cox RI. Steroid and phyto-oestrogen binding to sheep uterine receptors *in vitro*. J Endocrinology **52:**299–310, 1972.
6. Mathieson RA, Kitts WD. Binding of phyto-estrogens and oestradiol-17β by cytoplasmic receptors in the pituitary gland and hypothalamus of the ewe. J Endocrinol **85:**317–325, 1980.
7. Martin PM, Horwitz KB, Ryan DS, McGuire WL. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology **103:**1860–1867, 1978.

8. Cheng E, Story CD, Yoder L, Hale WH, Burroughs W. Estrogenic activity of isoflavone derivatives extracted and prepared from soybean oil meal. Science **118**:164–165, 1953.
9. Bickoff EM, Livingston AL, Hendrickson AP, Booth AN. Relative potencies of several estrogen-like compounds found in forages. J Agr Food Chem **10**(5):410–441, 1962.
10. Folman Y, Pope GS. The interaction in the immature mouse of potent oestrogens with coumestrol, genistein and other uterovaginotrophic compounds of low potency. J Endocrinol **34**:215–225, 1966.
11. Okura A, Arakawa H, Oka H, Yoshinari T, Monden Y. Effect of genistein on topoisomerase activity and on the growth of [VAL12] Ha-ras-transformed NIH 3T3 cells. Biochem Biophys Res Commun **157**:183–189, 1988.
12. Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S-I, Itoh N, Shibuya M, Fukami Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem **262**:5592–5595, 1987.
13. Faber KA, Hughes CL Jr. The effect of neonatal exposure to diethylstilbestrol, genistein and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. Biol Reprod **45**:649–653, 1991.
14. Barnes S, Grubbs C, Setchell KDR, Carlson J. Soybeans inhibit mammary tumors in models of breast cancer. In: Pariza M, ed. Mutagens and Carcinogens in the Diet. New York: Wiley-Liss, pp239–253, 1990.
15. Barnes S, Peterson TG, Grubbs C, Setchell KD. Potential role of dietary isoflavones in the prevention of cancer. In: Jacobs M, Ed. Diet and Cancer: Markers, Prevention and Treatment. New York: Plenum Press, pp133–145, 1994.
16. Hawrylewicz EJ, Huang HH, Blair WH. Dietary soybean isolate and methionine supplementation affect mammary tumor progression in rats. J Nutr **121**:1693–1698, 1991.
17. Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, Day NE. Dietary effects on breast cancer risk in Singapore. Lancet **337**:1197–1200, 1991.
18. Aldercreutz H, Honjo H, Higashi A, Fotsis T, Hamaten E, Hasegawa T, Okada H. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am J Clin Nutr **54**:1093–1100, 1991.
19. Banerjee MR, Wood BG, Lin FK, Crump LR. Organ culture of whole mammary gland of the mouse. Tissue Culture Assoc **2**:457–462, 1976.
20. Russo IH, Tewari M, Russo J. In: Jone TC, Konishi Y, Mohr U, Eds. Integument and Mammary Gland, Monograph Series on the Pathology of Laboratory Animals. Berlin: Springer-Verlag, 1988.
21. Russo IH, Russo J. Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenzanthracene. J Natl Cancer Inst **61**:1439–1449, 1978.
22. Russo J, Wilgus G, Russo IH. Susceptibility of the mammary gland to carcinogenesis I. Differentiation of the mammary gland as determinant of tumor incidence and type of lesion. Am J Pathol **96**:721–736, 1979.
23. Kokoska SM, Hardin JM, Grubbs CJ, Hsu C. The statistical analysis of cancer inhibition/promotion experiments. Anticancer Res **13**:1357–1364, 1993.
24. McEwen BS. Interactions between hormones and nerve tissue. Sci American **235**:48–58, 1976.