ORIGINAL RESEARCH

INDUCTION OF EXPERIMENTAL MAMMARY CARCINOGENESIS IN RATS WITH 7,12-DIMETHYLBENZ(A)ANTHRACENE

Alfredo Carlos S. D. Barros, Elisa Naomi K. Muranaka, Lincon Jo Mori, Christina Helena T. Pelizon, Kyoshi Iriya, Giovana Giocondo and José Aristodemo Pinotti

BARROS ACSD et al. Induction of experimental mammary carcinogenesis in rats with 7,12-dimethylbenz(a)anthracene. **Rev. Hosp. Clín. Fac. Med. S. Paulo 59**(5):257-261, 2004.

PURPOSE: To test an experimental model of chemical mammary carcinogenesis induction in rats.

METHODS: Twenty young virgin Sprague-Dawley female rats, aged 47 days, received 20 mg of 7,12-dimethylbenz(a)anthracene (DMBA) intragastrically by gavage. Afterwards, at 8 and 13 weeks, their mammary glands were examined. At the end of the experiment, the animals were sacrificed, and the mammary tumors were measured and weighed. Tumor fragments were analyzed using light microscopy.

RESULTS: Eight weeks after DMBA injection, 16 rats presented at least 1 breast tumor (80%). After 13 weeks, all of them (100%) developed breast carcinomas that were confirmed by histopathological analysis.

CONCLUSION: This experimental animal model of chemical mammary induced carcinogenesis is feasible and can be used in further experiments on the role of tumorigenic biomodulator substances.

KEY WORDS: **Breast cancer. Animal models. Carcinogens. DMBA-induced tumors. Rat mammary carcinogenesis.**

Breast cancer represents the most common neoplastic disease in females, accounting for up to one third of new diagnoses of women's cancer in certain regions of the world. In developing countries traditionally known for low incidence of breast cancer, increases in both incidence and mortality have been recently detected.[1]

The fundamental issue in breast cancer control is prevention (primary and secondary), which depends on identification of the determinants of the disease, in terms of initiation and promotion.

The possibility of using biomodulators of breast carcinogenesis, such as selective estrogen receptor modulators (SERMS), aromatase and cyclooxygenase inhibitors, and dietary factors is very promising.[2,3] Nevertheless, before clinical investigations are conducted, all of these strategies should be previously validated in animal models.

Mammary tumors arise spontaneously in a few animal species, for example dogs, rats, and mice.[4] For practical reasons most of the studies about experimental breast carcinogenesis are conducted with rodents.[5]

Mammary glands of several rat strains, mainly Sprague-Dawley and Wistar-Furth, are susceptible to transformation induced by chemical carcinogens, and the 2 most widely used active chemical inductors of mammary carcinogenesis are 7,12-dimethylbenz(a)anthracene (DMBA) and N-methylnitrosurea.[6,7]

Based on Russo and Russo experience,[8,9] we decided to test an animal model for chemical mammary carcinogenesis employing DMBA, given by gavage, in young female Sprague-Dawley rats.

The objectives of our study were to investigate the feasibility of the model, the percentage of rats that develop breast cancer, the number of glands affected per animal, and the histological aspects of the induced neoplasms.

From the Discipline of Gynecology, Hospital das Clínicas, Faculty of Medicine, University of São Paulo – São Paulo/SP, Brazil.
E-mail: clinab@terra.com.br
Received for publication on February 27, 2004.

METHODS

The group of test animals was formed by 20 young virgin Sprague-Dawley female rats. This type of rat lives an average of 3 years, starting its reproductive function, which lasts for about 1 year, at 50 to 60 days of age. They have 7 to 10 pregnancies during this period, delivering 7 to10 pups each time.

Mammary gland tumors were induced by a single dose of 20 mg of DMBA diluted in soy oil (1 mL) given intragastrically by gavage (Figure 1). All of the rats, with an average weight of 182.9 g (161-213 g), received the chemical carcinogen at the age of 47 days.

The animals were bred in our laboratory under ideal conditions of temperature, humidity, and light, and they were fed with appropriate ration in pellets and filtered water.

We performed physical examinations weekly. Each rat had 6 pairs of mammary glands that were checked by inspection, touching, and palpation. For evaluation of the induction pattern we performed 2 specific analyses at 8 and 13 weeks after drug injection.

At the age of 138 days (13 weeks after DMBA), the animals were sacrificed in a CO_2 chamber. Complete autopsies were performed, mammary tumors were measured and weighed, and the findings recorded. Representative fragments of the tumors were fixed in 10% formaldehyde and paraffin embedded. The blocks were sectioned every 5 mm, and slides were prepared with hematoxylin-eosin stain and examined using light microscopy.

RESULTS

The first assessment 8 weeks after DMBA administration revealed that 16 animals developed at least 1 mammary tumor (80%), with an average of 3.0 tumors per animal (0-6).

Thirteen weeks after DMBA administration, all of the rats (100%) developed breast tumors, with an average of 4.9 tumors per animal (1-15). The mean size of the tumors was 1.8 cm (0.5-5). The weight of the tumors ranged from 0.06 g to 29.3 g with a mean weight of 2.4 g (Table 1).

Figure 2 shows an animal with chemically induced breast tumors.

In 1 rat (animal 18), tumors were formed apart from the breasts in the neck area of the animal.

Histological examination showed that the neoplasms were adenocarcinomas with several morphological types. The most common type encountered was adenoid cystic carcinoma, which is characterized by sheets of tumor cells separated by small cystic spaces (Figure 3). Papillary carcinoma was also very common, which consists of proliferating epithelial cells with delicate cores of connective tissue separated by narrow spaces (Figure 4). A smaller proportion of tumors was of the myoepithelial type, characterized by a myxoid appearance and resembling mixed mammary tumors of dogs.

It is interesting to point out that, despite the tumor malignancy, no metastases were detected in the autopsies.

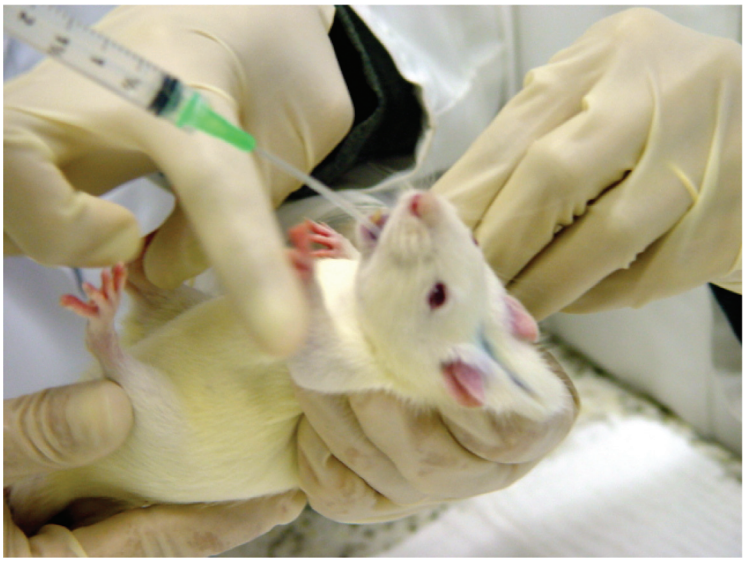

Figure 1 - Administration of DMBA by gavage.

DISCUSSION

The mammary gland is one of the few organs that is not totally developed at birth. It undergoes intense evolutive and functional modifications during puberty, pregnancy, and lactation. Russo and Russo[10] have described the developmental progression of human breast tissue and listed 4 different types of breast lobules. Type 1 (or virginal) is the most undifferentiated lobule and occurs in the immature female breast

Table 1 - Number of tumors per rat at 8 and 13 weeks after 7,12-dimethylbenz(a)anthracene (DMBA) administration.

ANIMAL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
8 weeks	1	1	3	1	_	2	2	_	_	1	4	4	7	6	2	6	1	7	_	1
13 weeks	7	6	3	1	1	3	3	1	3	5	5	5	7	8	4	8	4	15	6	4

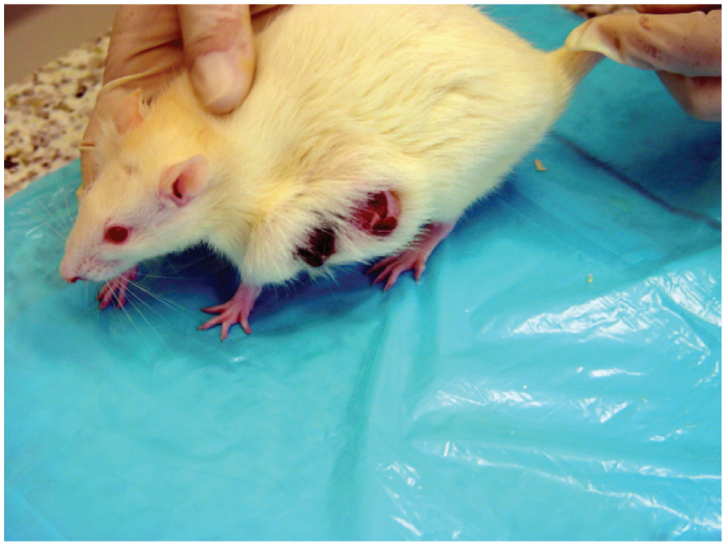

Figure 2 - Breast carcinomas induced by 7,12-dimethylbenz(a)anthracene (DMBA).

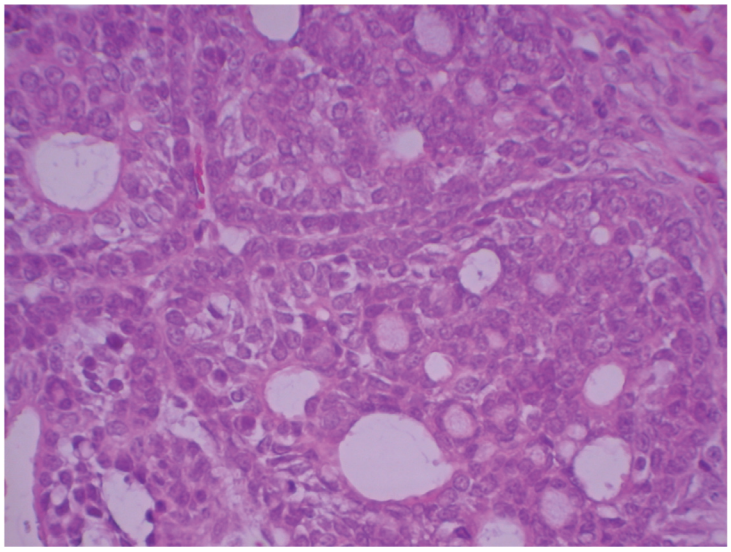

Figure 3 - Adenoid cystic carcinoma (HE-200x).

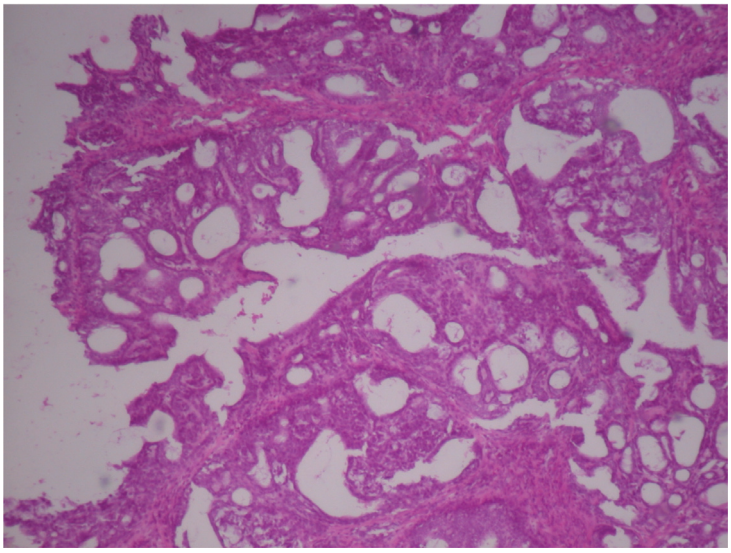

Figure 4 - Papillary carcinoma (HE-40x).

before menarche. Type 2 lobule has a more complex morphology, being composed by a higher number of ductular structures per lobule. Type 3 has an average of 80 alveoli (ductules) and is formed under gestational hormonal stimulation. Type 4 is a secreting lobule during lactation.

The mammary tumors in rats arise in the epithelium of the terminal end buds, which are comparable structures to the terminal ductal lobular units in the human breast.[12]

The degree of lobular differentiation is of importance in the susceptibility to carcinogenesis. Based on studies of the pathogenesis of human mammary cancer, it is possible to say that the type 1 lobule is the site of origin of preneoplastic lesions. Parous women undergo lobular differentiation, whereas nulliparous women seldom reach the type 3 lobule stage. The breasts of parous women free of cancer have the lowest percentage of type 1 lobules.

Lobules type 1 and 2 are characterized by having a shorter doubling time than type 3, growing faster and having a higher DNA labeling index.

The susceptibility of the mammary gland to DMBA carcinogenesis is strongly age-dependent, being maximal when the drug is administered to rats between the ages of 45 and 60 days, which is the age of the beginning of sexual maturity.[12] Active breast organogenesis and high rate of proliferation of type 1 and 2 lobules are characteristics of that period. The chance of chemically inducing breast cancer in rats is greater if DMBA is administered in this phase of the life of the animals. This was the reason why we injected the drug at the age of 47 days. According to the literature, the induced tumors are generally ductal carcinomas or papillary carcinomas, but it is possible that typical fibroadenomas, adenomas, and papillomas are also formed.[5,11,13]

Epithelial and myoepithelial cell proliferation were observed in most of the induced tumors in our experiment. Russo et al. found the same histological aspects. They also carefully presented the correlation between neoplastic and non-neoplastic alterations in the mammary gland of rats and in women, which was proposed in a consensus meeting on this subject held in Hannover, Germany, in 1987.[11] Most of the lesions found in rats have corresponding lesions in humans, allowing the translation of basic research in rats into the clinic.[14]

The induced DMBA tumors in this study were multifocal and locally aggressive, but no single case of metastases was identified. This fact is in agreement with studies done by other authors, and metastasis from even the most anaplastic induced tumors are low in frequency.[11]

The absence of metastases in chemically produced mammary neoplasms opens the door for speculation. According to Murad and von Haam,[13] the proliferation of epithelial and myoepithelial cells in relatively equal proportions may be a protective factor against metastasis. In human breast carcinomas, proliferation occurs almost exclusively in epithelial cells.

DMBA is highly lipophilic and requires metabolic activation for its carcinogenicity. Several tissues are capable of activating DMBA, and these include the mammary gland. In the breast, DMBA is converted to epoxides, active metabolites with a capacity for damaging the DNA molecule, the main event in carcinogenesis initiation. With the higher cellular proliferative index of types 1 and 2 lobules, there is higher metabolic activity and more epoxide formation.[15,16,17]

The first DMBA-induced tumor was observed about 5 weeks after injection of the carcinogen, but individual tumoral latency was not assessed in our study. It is possible, though, to grossly estimate tumor formation patterns, since after 8 weeks, there were tumors in 16 animals, and after 13 weeks, tumors were detected in all of them.

This experimental animal model closely mimics human breast cancer and can be used as a comparative group in further studies with the purpose of elucidating the role of biomodulation in mammary carcinogenesis. The effects of pretreatment and posttreatment of rats with hormones, antihormones, isoflavones, celecoxib, and many other substances that have action on mammary carcinogenesis will be the endpoints of future research in our laboratory using this animal model.

ACKNOWLEDGEMENT

This work was supported by FAPESP (grant 01/04169-2).

RESUMO

BARROS ACSD e col. Indução da carcinogênese mamária experimental em ratas com 7,12 – dimetilbenz(a)antraceno. **Rev. Hosp. Clín. Fac. Med. S. Paulo 59**(5):257-261, 2004.

OBJETIVO: Testar um modelo experimental de indução química de carcinogênese mamária em ratas.

MATERIAL E MÉTODOS: Com 47 dias de vida, 20 ratas Sprague-Dawley, jovens e virgens, receberam por gavagem intragástrica 20 mg de 7,12-dimetilbenz(a)antraceno (DMBA). Oito e 13 semanas depois da injeção de droga as mamas das ratas foram examinadas. Ao final os animais foram sacrificados e fragmentos dos tumores foram estudados ao microscópio.

RESULTADO: Oito semanas depois da injeção de DMBA 16 ratas apresentavam tumor nas mamas (80%). Com 13 semanas todas desenvolveram carcinomas de mama (100%), que foram confirmados por análise histopatológica.

CONCLUSÃO: Este modelo experimental de indução química de carcinogênese mamária é factível e pode ser empregado em futuras pesquisas para avaliar o papel de substâncias biomoduladoras da tumorigênese.

UNITERMOS: **Câncer de mama. Modelos animais. Carcinógenos. Tumores induzidos por DMBA. Carcinogênese mamária em ratas.**

REFERENCES

1. Jemal A, Thomas A, Murray T, Thun M. Cancer statistics 2002. CA Cancer J Clin 2002;52:23-47.

2. Powles TJ. Anti-oestrogenic chemoprevention of breast cancer-the need to progress. Eur J Cancer 2003;39:572-9.

3. Home LR,Dannenberg AJ. A role of cyclooxigenase-2 inhibitors in the prevention and treatment of cancer. Semin Oncol 2002;29(suppl11):111-19.

4. Nerurkar VR, Chitale AR, Jainapurkar BV, et al. Comparative pathology of canine mammary tumours. J Comp Pathol 1989;101:389-94.

5. Russo J, Russo IH. Experimentally induced mammary tumors in rats. Breast Cancer Res Treat 1996;39:7-20.

6. Dias MF, Sousa E, Cabrita S, Patricio J, Oliveira CF. Chemoprevention of DMBA induced mammary tumors in rats by a combined regimen of alpha tocopherol, selenium, and ascorbic acid. Breast J 2000;16:14-19.

7. Giullino PM, Pettingrew HM, Grantham FIT. N-nitrosomethylurea as mammary gland carcinogen in rats. J Natl Cancer Inst 1975;54:401-14.

8. Russo J. Russo IH. Biological and molecular bases of mammary carcinogens. Lab Invest 1987;57:112-37.

9. Russo IH, Russo J. Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenzan(a)thracene. J Natl Cancer Inst 1978;61:1439-42.

10. Russo J, Russo IH. Toward a physiological approach to breast cancer prevention. Cancer Epidemiol Biomark Prev 1994;3:353-64.

11. Russo J, Gusterson BA, Rogers AE, et al. Biology of disease: comparative study of human and rat mammary tumorigenesis. Lab Invest 1990;62:244-77.

12. Grubbs CJ, Juliana MM, Hill DL, Whitaker LM. Suppression by pregnancy of chemically induced preneoplastic cells of the rat mammary gland. Anticancer Res 1986;6:2395-401.

13. Murad TM, von Haam E. Studies on mammary carcinoma induced by 7,12-dimethylbenzanthracene administration. Cancer Res 1972;32:1404-15.

14. Clarke R. Animal models of breast cancer: experimental design and their use in nutrition and psychosocial research. Breast Cancer Res Treat 1997;46:117-33.

15. Clarke R. Issues in experimental design and endpoint analysis in the study of experimental cytotoxic agents in vivo in breast cancer and other models. Breast Cancer Res Treat 1997;46:255-78.

16. Russo J, Larref MH, Balogh G, et al. Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. J Steroid Biochem Mol Biol 2003;37:1-25.

17. Balogh GA, Russo IH, Russo J. Mutations in mismatch repairs genes are involved in te neoplastic transformation of human breast epithelial cells. Int J Oncol 2003;23:411-9.