

Benign and malignant mammary tumors induced by DMBA in female Wistar rats

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Summary

This study pretends to characterize 7, 12-dimethylbenz[a]anthracene-induced benign and malignant tumors. One hundred and twenty female Wistar rats were randomly allocated to two groups: Control Group and Induction Group; IG animals were given a single dose of DMBA and killed 24 weeks after. Other tumors besides breast tumors were diagnosed, mainly tumors of the salivary glands and ovarian benign epithelial tumors. Incidence of breast disorders was about 60%. Macroscopic mammary tumors varied in dimension from 2 mm to 55 mm. Malignant breast tumors (n=56) were essentially invasive ductal carcinomas (91.1%), G1 (92.2%), presenting histologic characteristics of good prognosis. Predominant benign breast disorders consisted of glandular (68.6%) and atypical (20%) hyperplasias reproducing histologic types of human breast diseases.

Different individual susceptibility to DMBA apparently occurs; while some rats never developed neoplasias, others exhibited several tumors.

Key words: Benign breast tumors; Breast cancer; Wistar rats; DMBA; Animal model.

Introduction

Experimental models of chemically-induced tumors have been widely used and remain very important in order to understand the potential of chemical agents, hormonal environment and dietary factors in the induction, promotion and/or the prevention of mammary carcinogenesis [1-4].

The mammary glands of several rat strains are susceptible to neoplastic transformation by chemical carcinogens, most notably Sprague-Dawley rats [5].

Animal models are multiple and carcinogens are various. Several animal models of chemically-induced mammary tumors have been described [6-10]. DMBA (7, 12-dimethylbenz[a]anthracene) is a potent carcinogen inducing numerous lesions in the rat mammary gland; several authors have described the susceptibility or refractoriness to mammary tumorigenesis [4, 11-13] and the biologic and molecular bases of mammary carcinogenesis [14-16].

Mammary adenocarcinoma induced in rats by means of chemical carcinogens, mainly DMBA, seems to provide a good model to better understand the multiple mechanisms of susceptibility to carcinogens [6, 11, 17, 18].

In the present animal model of carcinogenesis, development of mammary tumors follows classic pathways of initiation, promotion and tumor progression.

This study simulated a model of benign and malignant mammary tumors induced by DMBA in order to better

characterize developing tumors as not much information is available on benign mammary tumors and tumors of other organs and tissues when using DMBA in Wistar rats.

Material and Methods

One hundred and twenty outbred virgin female Wistar rats were obtained from the Instituto Gulbenkian de Ciência (Lisbon, Portugal). Animals were housed four to each cage, in a temperature (23±2°C) and humidity (50-55%)-controlled facility, on a 12 h light, 12 h dark cycle and fed standard laboratory chow. Food and water were available *ad libitum*.

At 50 days of age, rats were randomly allocated to two groups: Control Group (CG; n=20) and Induction Group (IG; n=100). Biopsies of the right cervical mammary gland were performed in all animals under general anesthesia (Ketamine i.m. 5 mg/100 g). CG animals received no drugs until the end of the experiment. IG animals were given a single dose of 7, 12-dimethylbenz[a]anthracene (DMBA) (65 mg/Kg) solvled in olive oil via an intragastric tube (FST 180061-50).

Both groups were kept under the described conditions and they were observed and palpated weekly to determine the development, localization and size of neoplasias. Animals were weighed monthly.

Twenty-four weeks after carcinogenic induction animals were killed by cervical dislocation. Rats that died before 24 weeks after DMBA administration were excluded from the study.

Macroscopic tumors were excised and size, measured in two perpendicular dimensions, was estimated from the mean diameter. Mammary glands free of palpable tumors were also removed and biopsies of several organs and tissues were performed in all rats: trachea, heart, lungs, esophagus, liver, spleen, pancreas, bowel, bladder, internal genital organs.

Tumors and tissue fragments were fixed in 10% buffered-formalin and a 5 µm sections were obtained from paraffin blocks

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and stained with H&E for histopathological examination. Breast disease pathology and respective histologic type were evaluated by application of the same pathologic criteria used for the classification of human tumors according to the International Histological Classification of Tumors by the World Health Organization. Grading system for breast cancer was the Scarff-Bloom-Richardson (SBR) classification [19, 20].

Results

CG rats were all alive and healthy at the end of the experiment. Ten rats from IG died before the end of the experiment; none presented benign or malignant tumors and the necropsy was not conclusive in determining any type of recognizable pathology. Those animals were excluded from the data.

Mammary gland biopsies performed before the carcinogenic induction (50 days of age) exhibited the same mammary developmental stage: terminal end buds (TEBs) as predominant mammary structures.

Total body weight remained similar in both groups of rats throughout the experimental period (Table 1). No statistically significant difference was observed when comparing body weight of tumor-bearing rats with that of healthy rats.

Other tumors besides breast tumors were diagnosed in IG animals. Table 2 shows the localization of benign and malignant tumors in IG rats. Mammary tumors were significantly more frequent than other types of tumors (35 benign breast tumors and 56 malignant carcinomas of this gland). The second more frequent localization of diagnosed tumors were salivary glands, presenting also benign and malignant tumors (adenomas and adenocarcinomas). Four ovarian tumors were diagnosed, all being benign epithelial tumors. A total of 49 benign tumors and 63 malignant tumors were diagnosed in this group.

Fifty-three (58.9%) animals in IG presented breast pathology; some presented mammary diseases affecting more than one gland. A total of 91 mammary tumors (benign and malignant tumors) were diagnosed considering the total amount of mammary glands.

In Table 3 we can see the main results of this study concerning total number of breast tumors, total number of rats with breast tumors and tumor incidence.

The mean diameter of macroscopic mammary tumors varied between 2 and 55 mm. All malignant breast tumors were macroscopic tumors; nevertheless, 30 (85.7%) benign mammary disorders were diagnosed after histologic examination of the glands as microscopic tumors.

Breast cancer was preferentially localized in cervical and thoracic mammary glands (n=38; 67.9%); abdominal and inguinal mammary glands presented only 18 (32.1%) malignant breast tumors.

Histologic examination of benign and malignant breast tumors is presented in Table 4. Invasive ductal carcinomas were classified according to the Scarff-Bloom-Richardson (SBR) classification. No grade 3 (G3) was found. Four breast cancers were classified as G2 (7.8%) and 47 (92.2%) as G1.

Table 1. — Rat body weight before DMBA administration (50 days old) and at the time of the necropsy (218 days old)

Rats (age)	CG (g)	IG (g)	p (Fisher)
50 days old	198±18 [159-251]	193±16 [152-246]	>0.05 (NS)
218 days old	321±42 [201-499]	314±40 [190-492]	>0.05 (NS)

Table 2. — Localization of benign and malignant tumors in IG rats

Tumors (localization)	Benign (n=49)	Malignant (n=63)
Mammary gland	35	56
Salivary gland	4	2
Ovary	6	0
Liver	2	0
Epiploon	1	0
Nose	1	0
Lymphatic system	0	2
Eye	0	1
Lung	0	1
Pancreas	0	1

Table 3. — Synthesis of the results concerning benign and malignant mammary tumors development after DMBA administration in IG rats

Tumors (localization)	Benign	Malignant
Rats with tumor (n)	27	32
Tumor incidence (%)	30	36
Total number of tumors (n)	35	56
Number of tumors per rat (X±2SD)	0.4±0.7	0.6±0.9
Number of tumors per tumor-bearing rat (X±2SD)	1.3±0.8	1.8±1.1
Dimension of tumors (X±2SD) (mm)	15±8	19±13
Body weight (X±2SD) (g)	316±32	313±45

Table 4. — Histologic classification of benign (n=35) and malignant (n=56) mammary tumors in IG rats. DCIS-ductal carcinoma in situ

	N	(%)
Malignant mammary tumors		
Invasive ductal carcinoma	51	(91.07)
DCIS	3	(5.36)
Other histologic types	2	(3.57)
Benign disorders of the mammary glands		
Glandular hyperplasia	24	(68.57)
Atypical hyperplasia	7	(20.00)
Fibroadenoma	3	(8.57)
Intraductal papilloma	1	(2.86)

Other histologic parameters such as tumor fibrosis, vascular density, vascular and lymphatic invasion and inflammatory cellularity were assessed and showed a low grade of invasiveness and aggressiveness so that these tumors could be classified as good prognostic neoplasias. No metastatic lesions were diagnosed in any biopsies performed of organs and tissues.

Discussion

The mammary gland is a major target of carcinogen induction by chemical carcinogens, namely DMBA, in several rodent strains [2, 8, 11, 15]. Usually mammary tumors arise rapidly and at high frequency following carcinogen administration. Nevertheless, several factors are able to modify the response of the gland to the carcinogen. It has been observed that the rodent strain [5, 13, 14, 21, 22], the animal's age by the time of carcinogen induction [3, 6, 7, 14, 17, 23-25], parity, lactation and other hormonal factors [3, 4, 11, 12, 26-29], dose and type of carcinogen [2, 7-10, 30], micronutrients, radiation, diet and drugs [31-35] have the potential to modify the susceptibility of the mammary gland to the carcinogenesis.

DMBA induces different lesions in the rat mammary gland. The morphology of these lesions appears to be related to the site of origin [7]. Several studies [6, 7, 11, 14, 17] have demonstrated that terminal end buds (TEBs) are preferential targets to DMBA effects (DMBA-DNA linking) in the neoplastic transformation of the mammary gland. The number of TEBs existing in the mammary gland by the time of DMBA administration is the most important factor in determining the incidence of adenocarcinomas [36, 37]. Biopsies performed at 50 days of age in our study clearly exhibited TEBs as predominantly mammary structures. All rats presented the same mammary gland developmental stage by the time of carcinogenic induction.

Previous studies [7, 11] have demonstrated that cervical and thoracic mammary glands of the female rat contained a larger amount of TEBs when compared with abdominal and inguinal mammary glands. This observation is comparable in our study in that there was a higher incidence of breast tumors in these sorts of glands.

Furthermore, this study demonstrates that Wistar rats are susceptible but of intermediate sensitivity to chemically-induced mammary tumors, having a tumor incidence of ~36%, less than the tumor incidence in Sprague-Dawley, Lewis and Fisher models [5, 21, 23].

Not much information is available on the carcinogenic effect of DMBA in other organs and tissues. Our results clearly demonstrate that the mammary gland is the preferential target for DMBA carcinogenic action; nevertheless, other benign and malignant tumors developed in this model. Besides breast disorders, rats developed malignant and benign neoplasias of the salivary glands (adenomas and adenocarcinomas), lymphomas and malignant tumors (adenocarcinomas) of the lungs, pancreas and eye (tumor not classified). Exclusively benign were ovarian tumors (benign epithelial tumors), tumors of the liver, epiploon and nose.

In about 96% of cases, malignant mammary tumors are ductal adenocarcinomas, the same histological type as the human tumors. According to the WHO classification of tumors and the grading system SBR classification, diagnosed mammary tumors were of poor potential in aggressiveness and invasiveness and could be classified

as good prognostic tumors. Besides, no metastatic lesion was diagnosed in all organs and tissues studied (Material and Methods).

No rat with breast cancer presented malnutrition while lymphoma rats presented a severe weight loss. Body weight of animals with mammary tumors did not differ significantly when compared with body weight of healthy rats.

Concerning benign breast tumors, our results indicate that both benign and malignant mammary tumors could occur synchronously in the same animal. On the other hand, some rats exhibited exclusively benign disorders. In the majority of cases, benign tumors were diagnosed histopathologically as they had no macroscopic appearance. They presented the same histological types as human benign breast tumors. Besides hyperplasias (glandular and atypical) rats also developed fibroadenomas and intraductal papillomas.

In summary, the results reported herein demonstrate that Wistar rats are susceptible to chemically-induced tumors, presenting an intermediate sensitivity. We have also shown that other types of benign and malignant tumors besides breast tumors developed in this animal model.

An interesting observation was that benign and malignant tumors of the mammary gland were diagnosed in different animals but could also occur in the same animal.

A practical question that needs to be addressed in the future is related to the issue of whether benign tumors are pre-neoplastic lesions or not and why this model of carcinogenesis is able to produce benign and malignant tumors in a synchronous way. All rats submitted to the carcinogen were from the same source, the same strain, maintained under homogeneous conditions and submitted to the same procedure of carcinogenic induction. It is difficult to explain why several rats never developed neoplasias while some of them developed two or more tumors and why rats could display benign disorders and malignant tumors at the same time. Further investigation is needed to understand these observations and answer these questions.

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