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Comparative aspects regarding MNU-induced mammary carcinogenesis in immature Sprague-Dowley and Whistar rats

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Abstract. The research carried out on an experimental mammary cancer model by using two breeds of rat, such as Sprague-Dawley (n=10) and Wistar (n=9) female rats. There have been used immature rats that received intraperitonealy a single dose of methyl-nitroso-urea (55 mg/kg MNU). The rats were kept in optimal conditions of temperature, food and hygiene for 8 months. The Wistar rats were sacrificed by deep narcosis with Halothane, and were harvested samples for histopathology exam. The Sprague-Dawley rats were not slaughtered being utilized still to test the efficacy of some antioxidative agents in mammary cancer prevention. In Wistar rats, mammary tumor incidence was reduced representing 33,3% of all cases. In 22,2% of all cases precancerous mammary lesions occurred. On the other hand, MNU generated a great variability of tumor types in various tissues and organs, such as: liposarcoma, nephroblastoma, bronchial gland carcinoma, hemangiosarcoma, squamous carcinoma, sebaceous gland carcinoma, small cell lung carcinoma and lipoma. Comparing to this, in Sprague-Dawley rats mammary tumor incidence following to MNU-intake was 80% of all cases. Concluding, the most appropriate breed of rat that could be utilized as experimental model to study canine and human mammary cancer is Sprague-Dawley.

Keywords. mammary tumor, Whistar rat, Sprague-Dowley rat, methyl-nitroso urea.

INTRODUCTION

Mammary cancers represent one of the main causes of dying in bitch, and because of similarity with breast cancer in women there are numerous studies regarding canine mammary tumors and breast cancer in women. The high incidence of mammary tumors in women and dogs determined scientific community to develop comparative models by realizing mammary tumor induction in laboratory animals. Conformal to specialty literature, mammary tumors could be induced using chemical carcinogen such as methyl-nitroso-urea (MNU), no matter of administration way (7-12). In numerous scientific reports have been used Sprague-Dawley rat breed. This breed seems to have a great susceptibility to develop mammary tumours following MNU intake (6, 7-12). Comparing with canine mammary cancer in which mammary tumours occurs in posterior abdominal or inguinal mammary glands (1), in Sprague-Dawley rats mammary tumours onset predominantly in the pectoral mammary glands (7-12).

There seems to be some differences regarding mammary tumor induction using MNU in some different immature rat breeds. According to this, in our study we realized an experimental model of mammary tumor induction in two breeds of rats, using chemical

carcinogen MNU. Also, there have been utilized Sprague-Dawley rats and Wistar rats in order to establish the most appropriate rat breed for MNU-induced mammary carcinogenesis.

MATERIALS AND METHODS

The study was conducted in accordance with the European Union legislation on animal experimentation. Wistar and Sprague-Dowley females and sexually immature 37 days old rats were used in actual experiment; the rats were obtained from the Cantacuzino Institute, Romania. The rats have been kept in controlled environmental conditions for temperature and humidity (22-23°C air temperature, approximate 60% humidity, and 12h light-dark cycle). The animals had free access to standard rodent pelleted diet (Cantacuzino Institute, Romania) and tap water. All procedures were performed according to the practices of the Romanian board of animal research, being also approved by the committee of animal ethics from Faculty of Veterinary medicine Cluj-Napoca, Romania.

N-methyl-N-nitroso-urea (MNU) was used to induce mammary tumours (Sigma Chemical Co., St. Louis, MO). MNU was dissolved immediately before use in 0,9% Na Cl. After quarantine 27 animals (fourteen Sprague-Dowley rats and thirteen Wistar rats) were randomly divided and housed in four groups as follow: group 1 Wistar rats inoculated with MNU (n=9), group 2 Sprague-Dowley rats inoculated with MNU (n=10), and group 3 and group 4 (n=4 each) representing control groups. The carcinogen was inoculated intraperitoneally in groups 1 and 2, respectively a single dose of 55 mg MNU/kg body weight. The females were controlled periodically (after 3 weeks of the MNU inoculation, the rats were palpated once/week) to detect the initial stages of pre-neoplastic and neoplastic mammary lesions. The Wistar rats were euthanized by cardiac blood extraction (after deep narcosis with halothane) at 8 months from intraperitoneal MNU administration. The necropsy revealed the gross features of the tumors, respectively the size, consistency, gross section aspect, tumor location, regional lymph nodes integrity. There were harvested samples from tumors for histopathology exam. Samples harvested for histopathology exam were fixed into 10% buffered formalin and processed using paraffin technique. Sections were stained by usual methods, such as Masson's trichrome and hematoxylin-eosin. The Sprague-Dawley rats were not slaughtered being utilized still to test the efficacy of some antioxidative agents in mammary cancer prevention.

RESULTS AND DISCUSSIONS

Administration of MNU induced no acute toxicity in treated rats. Concerning the group 1 represented by Wistar female rats (n=9) that received the carcinogen MNU, obtained data revealed that MNU induced few mammary tumors, respectively in about 33,3% (n=3) from all subjects of group 1 (n=9). All diagnosed mammary tumors were benign, such as: 2 simple adenomas and 1 mammary fibroadenoma. The tumors size varied from 0,2 to 6,5 cm. On the other hand we found dysplastic and hyperplastic lesions. Dysplastic mammary lesions were represented by cystic mastopathy and ductal ectasia in 22,2% from all cases. Regarding hyperplastic mammary lesions, in 22,2% of cases was diagnosed lobular hyperplasia, such as typical epitheliosis and simple adenosis. Despite of having reduced number of mammary precancerous and cancerous lesions, there was diagnosed an increased number of other tumor types in several organs. Also, we diagnosed a total of 21 tumors in group 1, and only 4 of them were benign and the rest of 17 were malign tumors. There have been diagnosed the following tumor types: bronchial gland carcinoma, sebaceous gland carcinoma,

nephroblastoma, liposarcoma, hemangiosarcoma, squamous carcinoma, small cell lung carcinoma, and lipoma. Mammary tumors were represented of two simple adenomas and one mammary fibroadenoma. It should be mentioned that all subjects developed at least one tumor. From all nine rats only three developed just one tumor, the rest of them having several.

Comparing with the group 1, in group 2 that was represented by Sprague-Dawley rats (n=10) inoculated with MNU, obtained data indicate a higher mammary tumour induction up to 80% (n=8) from all subjects of group 2 (n=10). Furthermore, there is a great variability between subjects regarding the number of mammary tumours/rat. Also, six rats developed 1 mammary tumour, one subject had 2 mammary tumours, and the last one had 5 mammary tumours in different mammary glands. There are two rats that did not developed already mammary tumours, but because the rats were not slaughtered, it is possible to have some tumors in some other tissues than mammary parenchyma. The total number of mammary tumours obtained in this group is 13 comparing with only 3 in group 1. Because the rats from group 2 were not slaughtered yet, we don't know if MNU induced some other tumours in various organs as we notice it in group 1. Regarding the mammary tumour size, there is a great variability ranging from 0,5 to 4,5 cm (Table 1.).

Tab. 1**.**

Comparative aspects regarding mammary tumor development in groups 1 and 2 inoculated with MNU.

	Mammary tumours incidence, size and location					
Rat nr.	**Group 1 (Wistar rats)**			**Group 2 (Sprague-Dawley rats)**		
	Tumor (+/-)	Size (cm)	Location	Tumor (+/-)	Size (cm)	Location
1	-	-	-	+	*2,5* *3,5*	*Pectoral (M1)* *Inguinal (M5)*
2	-	-	-	+	*3*	*Thoracic (M3)*
3	-	-	-	+	*1,5* *3* *3,5* *2* *0.5*	*Pectoral (M1)* *Thoracic (M3)* *Abdominal left (M4)* *Abdominal right (M4)* *Inguinal (M5)*
4	-	-	-	+	*1*	*Inguinal (M5)*
5	-	-	-	+	*3*	*Inguinal (M5)*
6	+	*0,5*	*Inghinal (M5)*	+	*4,5*	*Pectoral (M2)*
7	-	-	-	+	*1*	*Pectoral (M2)*
8	+	*6,5*	*Inghinal (M5)*	+	*1,5*	*Pectoral (M2)*
9	+	*0,3*	*Inghinal (M5)*	-	-	-
10				-	-	-

M1 to M5 – represents the number of mammary gland belonging to mammary chain.

The distribution of the tumours in mammary gland chain was as follows:

- first mammary gland (M_1) – 2 tumours (15,3%);
- thoracic mammary glands ($M_{2,3}$) – 5 tumours (38,4%);
- abdominal and inguinal mammary glands ($M_{4,5}$) – 6 tumours (46,1%).

In *table 1.* are presented the details regarding mammary tumours incidence, size, number and location in the two groups. It is strikingly clear that the most appropriate breed of rat which should be utilized as experimental model for the study of canine and human mammary cancer is Sprague-Dawley. As suggested in many studies that utilized MNU-induced mammary carcinogenesis in immature Sprague-Dowley rats, the tumours are arising predominantly in anterior mammas (M1 to M3) (7, 8). This aspect have been observed in our study too by having 53,7% mammary tumours arising in M1 to M3.

In our study the mammary tumor incidence in Whistar rats was 33,3%, comparative with bibliographic rapports that indicate values between 11.1-71.5% (4, 6, 7-12). Mammary tumor incidence in Whistar rats indicate low values of only 11.1-42%, comparatively with other rat lines (Sprague Dawley, Nobil, F 344, ACI/N) where the mammary tumor onset is higher (ranging from 71.5 to 95%) (4, 6, 7). Nevertheless, as could be observed in group 1, the Wistar rats can be quite useful as experimental model for a great variety of tumour types (bronchial gland carcinoma, sebaceous gland carcinoma, nephroblastoma, liposarcoma, hemangiosarcoma, squamous carcinoma, small cell lung carcinoma, and lipoma). The process of carcinogenesis is still debated by having numerous unknown aspects. According to this the Wistar rats represents the best experimental model for the study oncogenesis and carcinogenesis pathways in different tumor types.

There are many possibilities to induce mammary tumours, but bibliographic data indicate the MNU as election carcinogen comparing with DMBA (7,12-dimethylbenz(a)anthracene) (13). MNU have in some rapports 90-95% efficiency, no matter of administration way (intravenous, intraperitoneal, subcutaneous) (7-9), comparatively with DMBA (4). Our results suggest not only the utility of MNU as election candidate for chemically induced carcinogenesis, but reveal as well the importance of rat line (breed) in order to obtain mammary tumours. The present study indicates a great difference regarding mammary tumor incidence in two rat breeds, such as Wistar and Sprague-Dawley rats, by using MNU as carcinogen.

CONCLUSIONS

Mammary tumor induction determined by MNU was reduced in Wistar rats (33,3%) comparing with Sprague-Dawley rats (80%), suggesting the utility of the last rat line in the study of chemically-induced mammary carcinogenesis. Mammary tumours have been encountered in anterior mammas (53,7% in M1 to M3), and there were two subjects that had several mammary tumors in different mammary glands. Despite of having a reduced incidence of mammary tumours, the Whistar rats represents a useful experimental model for the study of carcinogenesis in various tumor types, in this group being encountered the following tumours: bronchial gland carcinoma, sebaceous gland carcinoma, nephroblastoma, liposarcoma, hemangiosarcoma, squamous carcinoma, small cell lung carcinoma, and lipoma. MNU represents the election candidate as carcinogen in rat experimental model, a single dose of 55 mg/kg MNU (intraperitoneally inoculated) being capable to initiate tumorigenesis.

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