HISTOPATHOLOGY OF MAMMARY TUMOURS IN FEMALE RATS TREATED WITH 1-METHYL-1-NITROSOUREA

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Objective. To induce, evaluate and classify advanced stages of mammary gland tumours induced by MNU.

Methods. Female Sprague-Dawley rats were injected intraperitoneally with 1-methyl-1-nitrosourea (MNU; 50 $mg.kg^{-1}$) on the day 33, 40, 47, 54 and 61 of age in the first experiment and on 50th and 113th day in the second experiment. On the 117th day (first experiment) and on the 153rd day of age (second experiment) the rats were sacrificed by decapitation and their mammary glands were evaluated both macroscopically and microscopically for the presence of grossly detectable mammary tumours. Mammary tumours were classified according to Russo et al. (1990).

Results and conclusions. The final incidence of palpable carcinomas was ranging from 60 % to 76 %. All microscopically evaluated tumours were malignant. Among the total number of lesions classified the percentage of invasive tumours ranged from 35 % to 44 %. No metastases were observed in other organs in MNU treated animals.

Key words: Histopathology – Mammary carcinoma – 1-methyl-1 Nitrosourea – Female rats

Since the histological structure of rat mammary gland tumours resembles those of human ones, the induction of mammary carcinomas by the application of 1-methyl-1-nitrosourea (MNU) to female rats is one of the most frequently used animal models for the investigation of breast carcinogenesis and mammary tumours treatment (Welsch 1985; Russo et al. 1990; Thompson et al. 1995). So far a considerable attention has been paid to the mouse models, despite the fact that the majority of the mouse lesions are alveolar, while in humans and rats they are predominantly ductal (Thompson et al. 1995). In rats the most of highly malignant tumours show some common features with intraductal and infiltrating ductal carcinomas in humans (Russo et al. 1990).

In 1975 Bots and Willighagen (cf. Welsch 1985) reported that 6 months after monthly intravenous injections of MNU to female Lewis rats multiple mammary carcinomas appeared. Such model shows several advantages as compared to the widely used aromatic hydrocarbons, e.g. 7,12-dimethylbenzanthracene (DMBA) or 3-methylcholantrene (Gullino et al. 1975; Rose et al. 1980; Mc Cormick et al. 1981). Both the MNU and the DMBA models include reliability of tumour induction, organ site specificity, tumours of ductal origin and predominantly carcinomatous histopatholological characterisation, tumours of varying hormone responsiveness, and the potential to examine tumour initiation and promotion processes (Thompson and Adlaka 1991). MNU induced mammary tumours appear to be more estrogen dependent than those induced by DMBA (Rose et al. 1980). The proportion of benign tumours induced by MNU is lower than that induced by DMBA (Gullino et al.1975). Mc Cormick et al. (1981) concluded that one of the major disadvantages of the DMBA model is the lack of tumour metastases. Generally, MNU induced mammary carcinomas are aggressive and locally invasive

Table 1
Histological classification of palpable tumours after MNU treatment (50 mg.kg^{-1}) (1st experiment)

Animal No.	Number of lesions	Histol. classification
1	1	IDC-PCrCo
	1	DCIS-CrCo
2	1	IDC-PCrCo
	1	IDC-CrCo
	1	IDC-PCr
	2	DCIS-PCr
	1	DCIS-PCo
	1	DCIS-CrCo
3	1	DCIS-CrP
	1	DCIS-Cr
4	1	IDC-P
	1	DCIS-PCr
	1	ITCCr
5	1	DCIS-P
	1	IDC-P
	1	DCIS-PCr
6	1	DCIS-PCr
	2	NTC

Table 2
Histological classification of palpable tumours after MNU treatment (50 mg.kg^{-1}) (2nd experiment)

Animal No.	Number of lesions	Histol. classification
1	1	IDC-PCr
	1	DCIS-Cr
2	1	DCIS-P
	2	IDC-PCrCo
3	2	IDC-CrCo
	1	DCIS-PCr
4	1	IDC-PCo
5	1	IDC-Cr
	2	DCIS-PCr
7	1	DCIS-P
8	2	DCIS-PCo
	1	DCIS-CrCo

Invasive – I, , ductal carcinoma – DC, ductal carcinoma in situ – DCIS, non-invasive – N, tubular carcinoma – TC, papillary – P, cribriform – Cr, comedo – Co,

and they are capable metastasize (Thompson and Adlaka 1991).

The aim of the present study was to induce, evaluate and classify the advanced stages of mammary gland tumours induced by MNU.

Materials and Methods

Animals. Female Sprague-Dawley rats were obtained from Charles-River farm at 21 days of age. The animals were housed 4-5 per cage and maintained at 23 ± 2 °C and 12 h light:dark cycle. They were fed standard laboratory diet and had access to water ad libitum.

Experiments. In the first experiment MNU (50 mg/kg) was injected i.p. on the day 33, 40, 47, 54 and 61 of age alternately through the left and right abdominal wall. The MNU was always dissolved immediately before use in 0.9 % NaCl adjusted to pH 4 with acetic acid. The solubility of MNU in water at room temperature was 1.4 % (w/v). The experiment was terminated on the 117th day of the animals age. In the second experiment the rats were treated with the same doses of MNU on the 50th and 113th day of age. The experiment was terminated on the 164th day of age. The animals of the shame treated control groups received 0.9 % NaCl solution adjusted to pH 4 with acetic acid only.

Examination of tumours. All animals were sacrificed by decapitation and their skin was examined through translucent light. The mammary glands were evaluated for the presence of grossly detectable mammary tumours and the dissected animals with tumours were photographed to provide identification record on the location and gross morphology of lesions (Fig.1). All palpable tumours were excised and fixed in 10 % buffered formol and processed for histopathological evaluation. Paraffin sections of the excised tissues were stained with hematoxylin-eosin and according to Gomori. Mammary tumours were classified as recommended Russo et al. (1990).

Results

All tumours observed at authopsy were encapsulated and of solid consistention. There was no gross evidence of acute toxicity after the administration of MNU. Final body weights of MNU treated animals were 12.8 % less in the 1st experiment and 16.4 % less in the second one when compared to control rats. The final incidence of palpable carcinomas in two experiments ranged from 60 to 76 %. All carcinomas were malig-

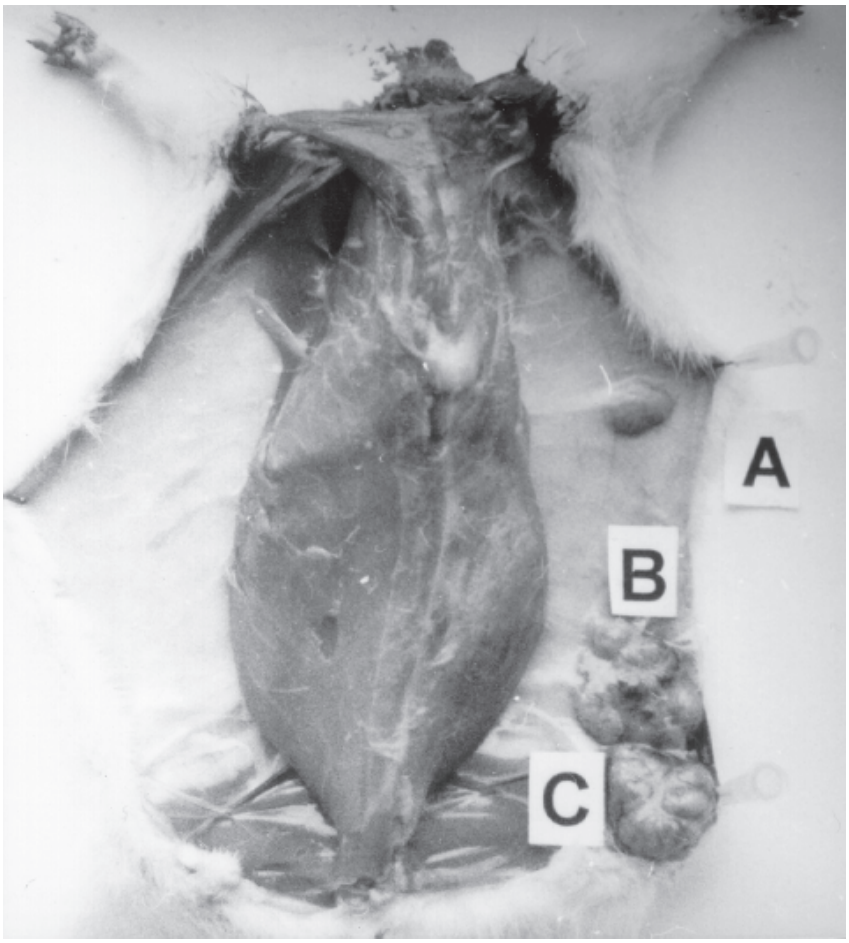

Fig. 1 Grossly detectable mammary tumours (A, B, C) after MNU treatment.

nant. There was higher variability in tumour incidence in the 2nd experiment, 2 animals did not develop any tumour after MNU treatment. Various combinations of papillary, cribriform or comedo patterns of ductal carcinomas occurred in tumours (Fig. 2). Tubular carcinomas appeared infrequently. The histological classification assigned to identified lesions of both experiments are shown in Tab. 1 and Tab. 2.

Of total number of lesions classified in the 1st experiment 35 % and in the second one 44 % were invasive. Carcinomas retained normal architecture of the gland and invaded surrounding tissues. The tissue invasion was mostly local. Some of invasive tumours were characterised by penetration of fingerlike projections or duct-like structures or solid sheets of epithelial cells into the surrounding stroma, while the others were characterised by breaked basement membrane (Fig. 3) and missed myoepithelium only. Massive stromal response demonstrated by fibrosis and mononuclear infiltration was frequently observed (Fig. 4). Individual neoplastic cells showed various degrees of neoplasia even in the same tumour. Epithelial cells were enlarged with increased nucleus/cytoplasm ratio and with enlarged nucleoli. No metastases in distant sites were observed.

Discussion

Intraperitoneal administration of MNU provides an extremely simple technic for inducing mammary carcinomas that improve the reproducibility, while the variation in tumor number per rat within a MNU dose

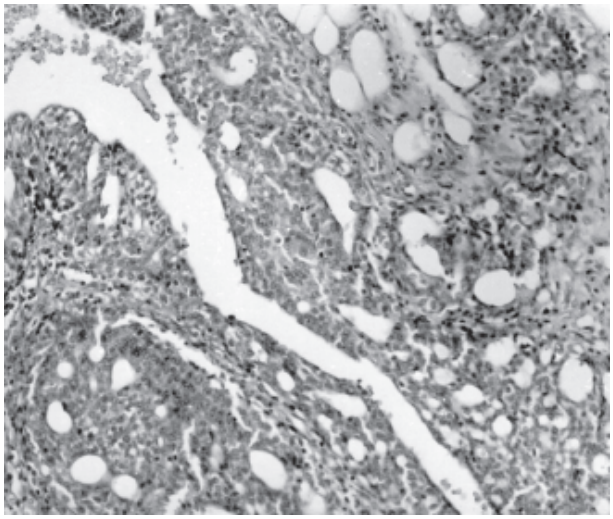

Fig. 2 Ductal carcinoma – papillary, cribriform pattern and necrosis, haematoxylin-eosin (x 100)

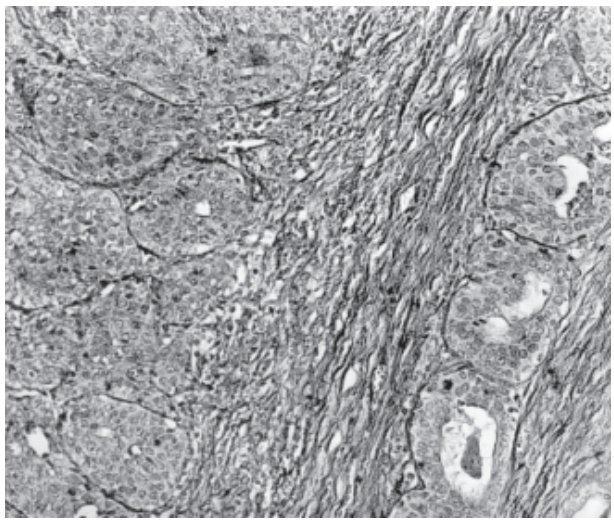

Fig. 3 Breaked basement membrane, Gomori (x 100)

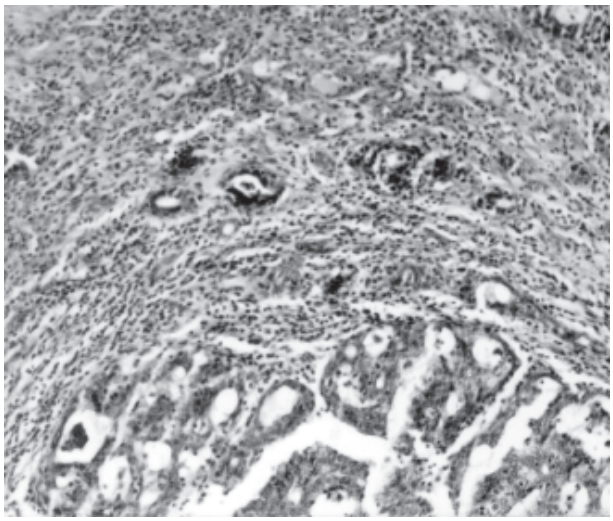

Fig. 4 Stromal response with mononucleare infiltration and penetration of duct-like structures or solid sheets of epithelial cells into the surrouding stroma, haematoxylin-eosine (x 100)

group is approximately 20-25% lower as compared to the intravenous or subcutaneous administration (Thompson and Adlaka 1991). Thompson et al. (1995) administered Sprague-Dawley rats with MNU (50 mg/kg) on the 21st day of age, because the proliferative activity of terminal end bud (TEB) is the highest in rats of average age of 20 days (Russo et al. 1992). High proliferative index is associated with the susceptibility to carcinogenic initiation (Russo et al. 1979). The cells initiated when MNU was given to 21 day old females were developing into ductal structures in the absence of a functional ovary (Thompson et al. 1995). TEB continues the extension into the mammary gland fat pad at this age even in ovariectomized animals (Sinha et al. 1983). Nandi et al. (1995) reported that greater proportion of mammary carcinomas induced in 21-day old rats could be hormone independent. Thompson et al. (1998) concluded that carcinomas in rats after treatment with MNU on the 21st day of age are dependent on ovarian hormones for their maintenance and growth even though the biological characteristic of these tumors could be considerably different from the tumours occurring in rats injected with carcinogen after ovarian function has been established (Thompson et al. 1995).

Endocrine status of MNU induced rat mammary carcinomas appear similar to human breast cancer (Russo et all. 1989). Prolactin and estrogen receptors are present in some of the MNU induced carcinomas (Rose et al. 1980). Prolactin is important in the early developmental stages of MNU induced rat mammary gland tumorigenesis as well (Welsch 1985). Progesterone at moderate levels commencing some days after or before MNU treatment inhibits tumor develop-

ment, when the steroid is administered during the time of MNU administration. This resulted in an enhancement of the incidence of mammary gland tumor. Pregnancy with lactation, commencing after MNU treatment suppressed the development of mammary carcinomas, although mean latency period was significantly reduced in the pregnant animals (Grubbs et al. 1983). Despite the significance of this issue, there is little known about the mechanism of the hormonal requirements of mammary carcinomas (Nandi et al. 1995). Thompson et al. (1998) found that in 21-day old male Sprague-Dawley rats after MNU injection both ovarian hormone dependent and independent mammary carcinomas can be induced. These authors also confirmed the hypothesis that the progression of pre-malignant to malignant lesions is inhibited in the mammary gland by ovariectomy and the hormone independent phenotype can be conferred at the time of carcinogenic initiation. Greater prevalence of the hormone independent phenotype would occur in the rats initiated with MNU prior to sexual maturation. The biological characteristic of these tumours could be considerably different from tumours occurring in rats injected with carcinogen after ovarian function has been established (Thompson et al. 1995).

We started with MNU administration after the established ovarian function in experimental animals. The first experiment was terminated on the 56th day after the last injection and the second experiment on the 51st day after the last injection of MNU. Due to our future intention on research work we focused our attention on the palpable tumours only. Therefore we decided to classify histologicaly the tumours they were over 3 mm diameter.

The most highly malignant tumours in rats have some common features with intraductal and infiltrating ductal carcinomas in humans, however they are a minority of tumours induced by the commonly used regimes. The abnormal epithelium usually remains rigidly confined by the adjacent stroma, shows no clear evidence of invasion and varies considerably within and between tumours. All of these features complicate the distinction between benign and malignant lesions (Russo et al.1990).

During tumour progression in rat the preferential amplification of the mutated Ha-ras allele (GGA -> GAA, 12th codon), cyclin D1 gene (PRAD-1), insulin-like growth factor gene (IGF 2), loss of expression of the mitogenic growth factor gene, heparin-binding growth factor midkine gene (MK) and mutation in the tumour supressor gene, p53, are seen in the mammary tumours (Sukumar et al.1995; Chen et al. 1996).

In experimental model of mammary carcinogenesis in female Sprague-Dawley rats induced by two intrajugular injection of MNU (50 mg/kg), beginning at 44-49 days of age, the acumulation of p53 protein in cell was demonstrated in 22 from 37 rat mammary tumours. These results indicate that elevated cellular content of p53 is a common event in invasive palpable mammary tumours induced by MNU in this model system (Crist 1996).

Lu et al. (1997) demonstrated the overexpression of cDNA fragments of gene transcripts: rat homologues of human galectin-7 gene, the human/mouse melanoma inhibitory activity/bovine chondrocyte-derived retinoic acid sensitive protein gene, the mouse stearoyl-CoA desaturase-2 gene, and the mouse endo B cytokeratin/human cytokeratin-18 gene in MNU-induced rat mammary carcinogenesis model. These genes may represent mammary carcinoma-specific molecular markers that may be helpful in investigation of mammary carcinogenesis and its prevention.

In our study the stromal response very often observed in carcinomas was more prominent in invasive malignant lesions than in non-invasive or benign ones. The most typical and frequent of the MNU induced are papillary carcinomas (Russo et al.1990). Various combinations of papillary, cribriform or comedo paterns of carcinomas occurred in our study. Papillary carcinomas in uniform pattern were observed rarely, but they were often dominant in combinations with other components.

Mc Cormick et al. (1981) concluded that one of the advantages in the MNU model should be spreading of metastases to distant sites. These authors in lifetime dose response study after MNU have found non-mammary tumours in 10 % animals. Total tumour response consisted of over 400 mammary cancers, 88 benign mammary tumours, and 18 tumours in other organs. In our study all palpable lesions were malignant and most of them were invasive, but no metastasis was observed in other sites in our MNU treated animals. Rose et al. (1980) have provided biological characteristics of MNU induced tumors in Sprague-Dawley rats and they have been unable to demonstrate the occurrence of systemic metastases. Greaves and Faccini (1984) reported that

metastatic spread of most rodent neoplasia is uncommon and Russo et al. (1990) concluded that very few authors reported finding of metastases from mammary tumours generally. Similarly, Van Zweiten (1984) mentioned that metastases from even the most anaplastic tumours are at low frequency.

In conclusion, all classified carcinomas were malignant. In the 1st experiment 35 % and in the second one 44 % were invasive. Final body weights of MNU treated animals were 12,8 % less in the 1st experiment and 16,4% less in the second one when compared to control rats.

Acknowledgements

This work has been partly supported by the VEGA Grant No. 2/6085/99.

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Accepted: March 15, 2000