Eur J Cancer Clin Oncol, Vol. 18, No. 6, pp. 559–564, 1982.
Printed in Great Britain.

0277-5379/82/060559-06$03.00/0

Sequential Morphological Studies of the Esophageal Carcinoma of Rats Induced by *N*-Methyl-*N*-Amylnitrosamine*

K. SASAJIMA, Y. TANIGUCHI, S. OKAZAKI, K. MORINO, K. TAKUBO, K. YAMASHITA and A. SHIROTA
First Department of Surgery, Nippon Medical School, Sendagi 1-1-5, Bunkyo-ku, Tokyo and Department of Pathology, Saitama Cancer Center Research Institute, Ina-machi, Saitama-ken, Japan

Abstract—*N-Methyl-N-amylnitrosamine was given to male Wistar rats in the drinking water at a concentration of 0.003% for 8 weeks. Three rats were autopsied weekly from the first to the 25th week. The stages of hyperplasia, dysplasia and squamous cell carcinoma in the esophagus were chronologically observed. Hyperplasia and dysplasia were found by the 6th week and continuously observed until the end of the experiment. Early squamous cell carcinomas were found in the 13th week. They showed endophytic or exophytic growths. Histologically, the endophytic growths were well-differentiated squamous cell carcinomas and the exophytic growths were poorly-differentiated. Advanced carcinomas were first found in the 15th week and were almost well-differentiated. Carcinomas were not found in papillomas. The incidence of carcinomas increased with time, being 9 out of 15 rats (60%) in the final 21st through 25th weeks. Lymph node metastasis was found in one rat, killed at the 24th week.*

INTRODUCTION

THE CARCINOGENICITY of various kinds of nitroso compounds has been reported since the initial discovery by Druckrey *et al.* in 1961 [1]. Esophageal squamous cell carcinomas have also been induced by various alkyl-nitrosamines [2]. It was recently reported that oral administration of *N*-methyl-*N*-amylnitrosamine (MNAN) specifically induced carcinoma of the esophagus, although the period of administration was much shorter than that of other alkyl-nitrosamines [3]. Furthermore, the carcinogenicity of MNAN injected intraperitoneally at different doses was reported [4].

The histogenesis of esophageal squamous cell carcinoma in rats has been reported by many investigators. This carcinoma develops from hyperplasia and/or not through papilloma. Napalkov and Pozharrisski [5] reported that this carcinoma developed mainly in papilloma. However, the precancerous change reported was hyperplasia of the epithelium [6–8]. One of our aims has been to clarify these relationships through studies on carcinogenesis of the esophagus. That is, we have examined the relationship between the early stage of the carcinoma and the invasive carcinoma.

This paper reports the sequential morphological changes in the esophagus of rats following oral administration of MNAN.

MATERIALS AND METHODS

Six-week-old male Wistar strain rats weighing about 170 g were purchased from Nihon Rat Co., Ltd., Saitama, Japan. The rats were kept in the metal cages and the room was kept in the standard laboratory conditions. They were fed on CE-2 chow obtained from Nihon CLEA Inc., Tokyo, Japan. *N*-Methyl-*N*-amylnitrosamine (MNAN) was purchased from Izumi Chemical Laboratory, Kamakura, Japan. It was dissolved in deionized water to give a stock solution having a concentration of 0.3% and stored at 4°C in a dark room. The stock solution was diluted to 0.003% every other day, just before it was given to the rats. The rats received a 0.003% MNAN solution for the first 8 weeks, and from the 9th week they were given tap water. From the begining of the experiment, including the first 8 weeks, three rats were killed and autopsied weekly. In all

Accepted 18 December 1981.
*This work was partly supported by Grant-in-Aid (55-6) for Cancer Research from the Ministry of Health and Welfare, Japan.

cases, the tongue, pharynx, esophagus and stomach were removed as a mass from each rat. The stomach was opened along the greater curvature and the esophagus was opened longitudinally in the anterior wall. They were flattened on a cork board and were fixed with a 10% neutralized formalin solution. The whole esophagus was cut into three portions, the upper, middle and lower regions. Each portion was divided longitudinally into two strips. Six strips were embedded in paraffin wax and 3μm-thick sections were made. The sections were routinely stained with hematoxylin and eosin. Some selected samples were stained with periodic acid–methenamine and silver. These and other organs were examined carefully.

RESULTS

The body weight of rats increased up to the 15th week and then decreased after the 20th week. All tumors were seen in the esophagus, except one papilloma of the tongue. No special pathological changes were found in the forestomach. The changes induced in the esophageal epithelium by MNAN were ulceration, hyperplasia, dysplasia, papilloma and squamous cell carcinoma.

Ulceration

Erosion and ulceration with neutrophil infiltration were found focally in a few rats from the first to the 4th weeks. Erosion rarely developed after the 5th week.

Hyperplasia and dysplasia

Focal hyperplasia, thickening of the stratified squamous epithelium (Fig. 1), was found within the 5th week. Dysplasia, which means hyperplasia with structural and cellular atypia of the basal and spinous cells, was found in the 6th week. This was continuously observed until the end of the experiment.

Papilloma

Papilloma first developed in the 7th week and consisted of extensive, epithelial, finger-like projections which contained a connective tissue core. No squamous cell carcinoma was found in the papillomas, although they increased in size by the end of the experiment.

Early squamous cell carcinoma

The first early carcinoma was seen in the 13th week. The early carcinomas included carcinoma *in situ* and carcinomas which penetrated the basal membrane and invaded the submucosa. Two types of early carcinoma were observed. The endophytic type was a flat elevated lesion and was almost covered with mild hyperplastic epithelium. The tumor cells invaded the submucosa from the depressed area of the top of the tumor. The carcinomas were all well-differentiated and keratinized (Fig. 2). The exophytic type was developed suddenly from hyperplasia and made a flat tumor. The tumor cells had large, pyknotic nuclei and were less keratinized. They showed intra-epithelial growth and poor differentiation (Fig. 3).

Advanced carcinoma

Advanced carcinomas, invading the muscle layer and adventitia, were found at the 15th week. They were polypoid and well-differentiated (Fig. 4). Metastasis to the regional lymph node and invasion of the trachea were seen in one rat, sacrificed at the end of the 24th week (Fig. 5).

Incidence and number of tumors at various periods

The incidence and number of papillomas and carcinomas at the various periods are summarized in Table 1. Papillomas accounted for 79% and carcinomas for 21% of the total 156 tumors. The incidence of papillomas was higher in the 11th to 15th weeks than in the 6th to 10th weeks, but no differences were found after the 15th week. However, carcinomas were periodically increased. The incidence of carcinomas was 9 out of 15 rats in the 21st to 25th weeks.

DISCUSSION

It has been reported that alkyl-nitrosamines administered by various routes induced tumors of the esophagus in rats. Intraperitoneal injection of MNAN induced tumors of the nasal cavity and the trachea [4]. However, oral administration of MNAN specifically induced esophageal tumors and this was examined autoradiographically by using [^{14}C]-labeled MNAN [3]. The metabolism of MNAN is still unknown.

In the present study, oral administration of MNAN induced mostly esophageal tumors in a short time. The administration period was also short and the concentration of MNAN was low. It was interesting that *N*-methyl-*N*-amyl-nitrosamine induced papillomas and carcinomas, but diethyl-nitrosamine induced mostly carcinomas [7]. This suggested that MNAN is less carcinogenic than diethyl-

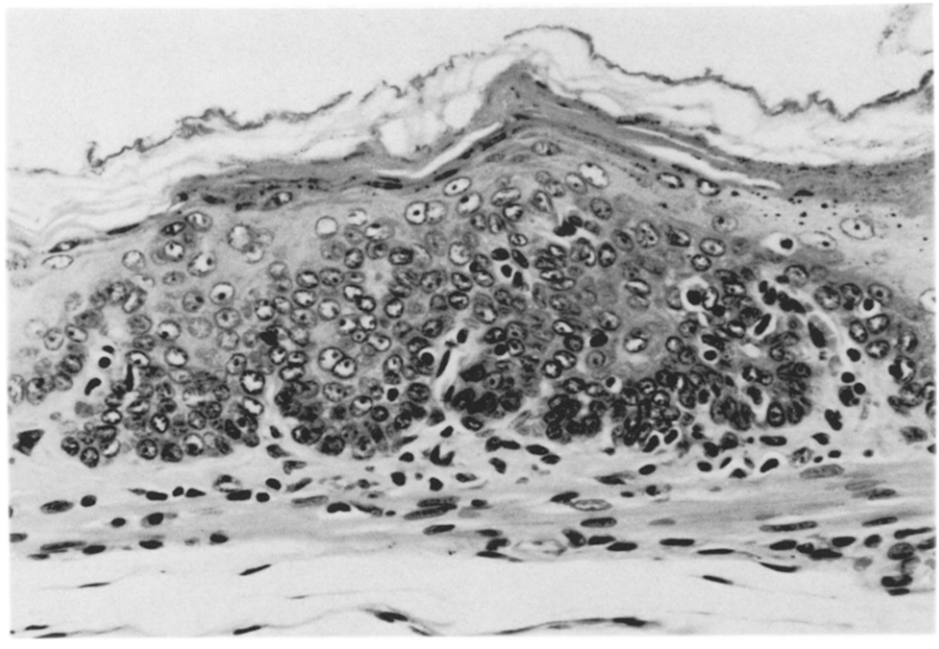

Fig. 1. *Hyperplasia and foci of proliferating cells in the basal layer of the esophagus. H.E.* ×100.

Fig. 2. *Early, well-differentiated squamous cell carcinoma of the esophagus. H.E.* ×40.

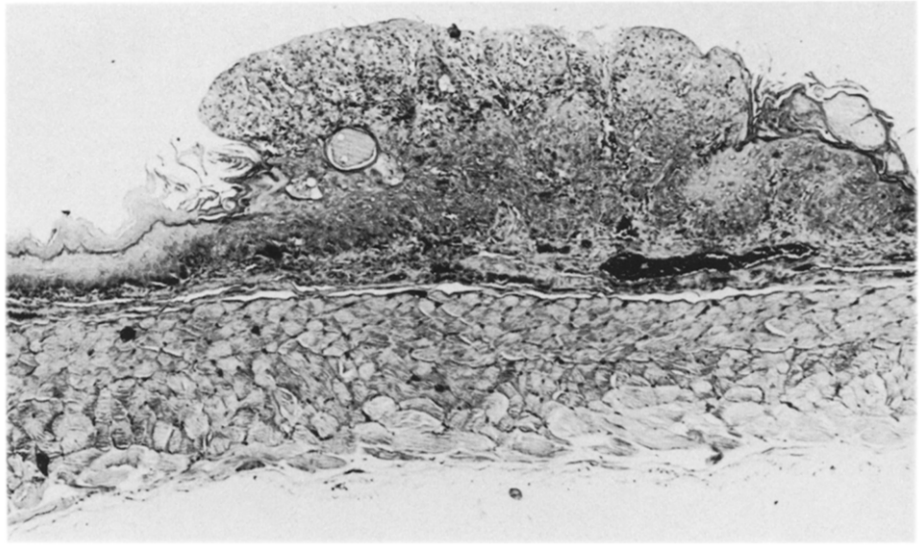

Fig. 3. Early, poorly-differentiated squamous cell carcinoma of the esophagus. H.E. ×40.

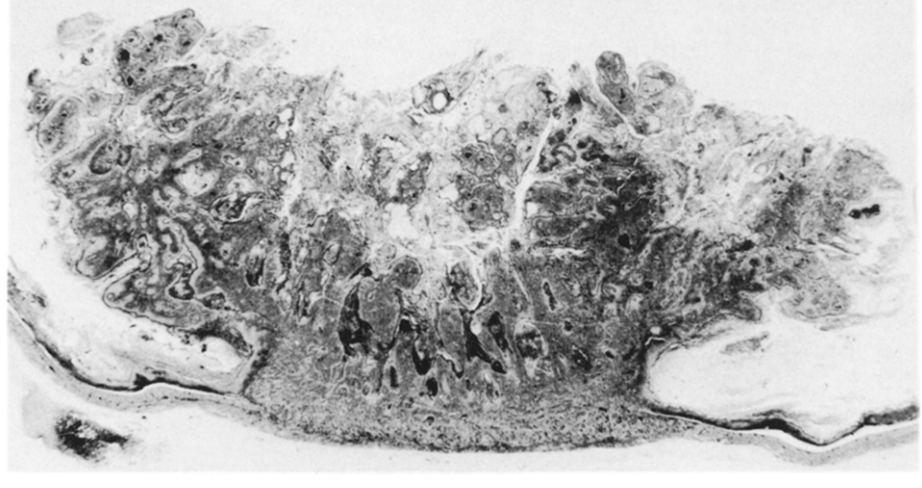

Fig. 4. Advanced, well-differentiated carcinoma of the esophagus. The tumor cells invaded into muscle layer. H.E. ×4.

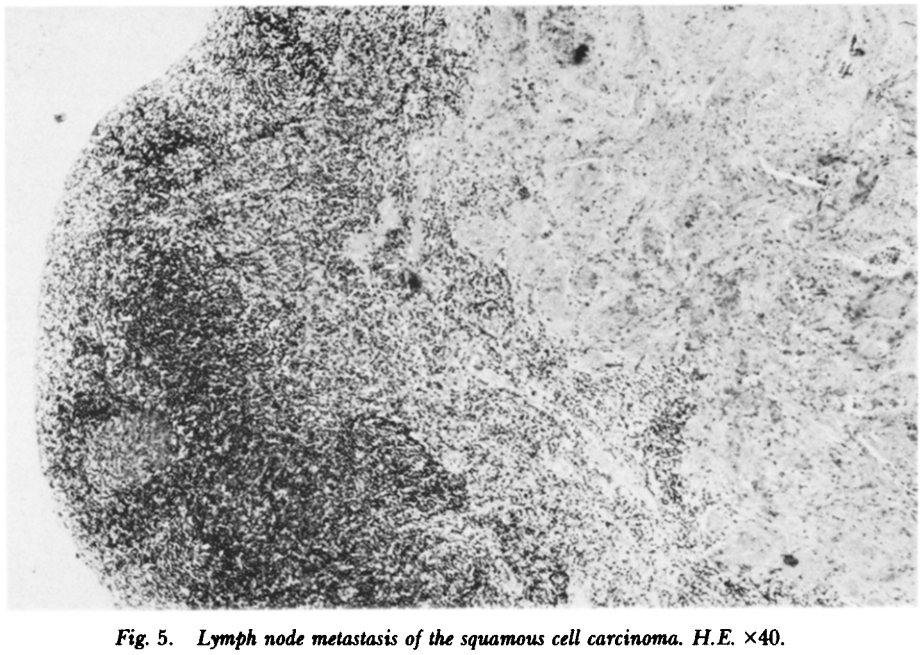

Fig. 5. Lymph node metastasis of the squamous cell carcinoma. H.E. ×40.

Table 1. *Incidence and number of papilloma and carcinoma of rats at various periods*

Weeks	Tumors (No. of rats)	Papilloma*	Squamous cell carcinoma†		
			Early	Advanced	Total
1–5	15	0/0	0/0	0/0	0/0
6–10	15	9/6	0/0	0/0	0/0
11–15	15	34/13	2/2	2/1	4/3
16–20	15	44/14	9/5	2/2	11/6
21–25	15	36/14	9/8	9/7	18/9

*Results given as number of papillomas and rats.
†Results given as number of carcinomas and rats.

nitrosamine in relation to the rat esophagus. A similar observation was made regarding the rat stomach. *N*-Propyl-*N'*-nitro-*N*-nitrosoguanidine, a propyl derivative of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and weak gastric carcinogen, induced intestinal metaplasia and adenocarcinoma in the rat stomach [9]. Intestinal metaplasia, thought to be a precancerous change in the human gastric carcinoma, was rarely found to be caused by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Oral administration of MNAN at a concentration of 0.003% for 8 weeks was considered a suitable condition for induction of esophageal squamous cell carcinoma in rats.

The sequential morphological changes caused in the esophagus by MNAN were ulceration, hyperplasia, dysplasia, papilloma and squamous cell carcinoma. MNAN, even at low concentration, was ulcerogenic, the same as other chemical carcinogens. We felt that hyperplasia and dysplasia might be precancerous changes of esophageal carcinoma and this has been confirmed by many investigators. Ito *et al.* [6] reported that papillomas consisted of reversible changes. In our experiment papillomas developed just after hyperplasia and in almost all the rats after the 11th week, but the number of papillomas did not change. Furthermore, no carcinomatous lesions were found in the papillomas at the termination of the experiment. We found two types of early carcinoma. The endophytic type was surrounded by hyperplastic epithelium and developed from the top of the tumor. It is thought that reversal of the stratified epithelium may be the cause, followed by growth in the submucosa; this shows a well-differentiated form. On the other hand, the early exophytic type developed abruptly from hyperplasia. The transformed cells showed intraepithelial growth. This type showed less differentiation than the endophytic type. Advanced carcinoma might be the result of progression of an early endophytic carcinoma. The growth of the endophytic carcinoma may be more rapid than that of the exophytic carcinoma. Sessile papillary carcinoma, reported by Stinson *et al.* [8], was considered to be another type of exophytic epithelial proliferation.

Longer term observations are necessary for detection of advanced, poorly-differentiated carcinomas and papilloma-derived carcinomas.

REFERENCES

1. DRUCKREY H, PREUSSMANN R, SCHMÄHL D, BLUM G. Carcinogene Wirkung von *N*-Methyl-*N*-nitroso-Anillin. *Naturwissenschaften* 1961, **48**, 722–723.
2. DRUCKREY H, PREUSSMANN R, IVANKOVIC S, SCHMÄHL D. Organotrope carcinogene Wirkung bei 65 verschiedenen *N*-Nitroso-Verbindungen an BD-Ratten. *Z Krebsforsch* 1967, **69**, 103–201.
3. IIZUKA T, ICHIMURA S, KAWACHI T. Esophageal carcinoma in rats induced by *N*-amyl-*N*-methylnitrosamine. *Gann* 1980, **71**, 94–99.
4. BULAY O, MIRVISH SS. Carcinogenesis in rat esophagus by intraperitoneal injection of different doses of methyl-*n*-amyl-nitrosamine. *Cancer Res* 1979, **39**, 3644–3646.
5. NAPALKOV NP, POZHARRISSKI KM. Morphogenesis of experimental tumors of the esophagus. *J Natl Cancer Inst* 1969, **42**, 922–940.

6. ITO N, KAMAMOTO Y, HIASA Y *et al.* Histopathological and ultrastructural studies on esophageal tumors in rats treated with *N*-nitrosopiperidine. *Gann* 1971, **62**, 445–451.
7. REUBER MD. Histopathology of preneoplastic and neoplastic lesions of the esophagus in BUF rats ingesting diethyl-nitrosamine. *J Natl Cancer Inst* 1977, **61**, 1471–1475
8. STINSON SF, SQUIRE RA, SPORN MB. Pathology of esophageal neoplasms and associated proliferative lesions induced in rats by *N*-methyl-*N*-benzylnitrosamine. *J Natl Cancer Inst* 1978, **61**, 1471–1475.
9. SASAJIMA K, KAWACHI T, MATSUKURA N, SANO T, SUGIMURA T. Intestinal metaplasia and adenocarcinoma induced in the stomach of rats by *N*-propyl-*N'*-nitro-*N*-nitrosoguanidine. *J Cancer Res Clin Oncol* 1979, **94**, 201–206.