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Induction of Adenocarcinomas in the Glandular Stomach of BALB/c Mice Treated with N-Methyl-N-nitrosourea

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Male 6-week-old BALB/c strain animals (groups 1 and 2) received 10 weekly intragastric intubations of 0.5 mg/mouse of N-methyl-N-nitrosourea. At week 11 the forestomachs were resected in group 1 but not group 2. Although many animals in group 2 died due to development of squamous cell carcinomas in the forestomach, development of cancers in the glandular stomach was quite similar in both groups. Well-differentiated adenocarcinomas in groups 1 and 2 were found at low incidence at week 20, rising to 100% at week 40, with two lesions metastasizing to the lymph nodes. Four poorly differentiated adenocarcinomas and 5 signet ring cell carcinomas were also found in 27 glandular stomach tumor-bearing animals.

Key words: N-Methyl-N-nitrosourea — Glandular stomach cancer — Mouse — Metastasis

Since 1967, when a high rate of induction of adenocarcinomas in the glandular stomach of rats with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG[2])[1] was described by Sugimura and his co-workers[2,3] this model has provided a powerful tool for experimental studies of gastric cancer. MNNG also induces stomach adenocarcinomas in hamsters[4] and dogs.[5] However, the glandular stomach of mice was generally found to be resistant to carcinogenic action[6] and several attempts have been made to establish a good alternative experimental model in this species.[7,8] While intragastric instillation of 4-nitroquinoline 1-oxide or 4-hydroxyaminoquinoline 1-oxide did cause adenocarcinomas in the glandular stomach of mice, the mortality rates were high and the cancer incidences were only low. Recently, localized X-irradiation of hypocatalasemic mice (a mutant of the C3H strain) was found to induce well differentiated gastric adenocarcinomas and signet ring cell carcinomas at high incidences, but with the disadvantage of heavy mortality due to stenosis of the gastrointestinal tract or infection.[8] Establishment of an appropriate experimental model of glandular stomach carcinogenesis induced by chemical carcinogens in mice therefore remains important, especially in view of the advantages mutant mice, transgenic mice and chimeric mice can bring to the analysis of molecular events. In the present study, we attempted to establish a new experimental model of mouse glandular stomach using N-methyl-N-nitrosourea (MNU) as the carcinogen.

Male BALB/c mice (Charles River Japan Inc., Kanagawa) 6 weeks old, were housed in plastic cages on hard wood chip bedding in an air-conditioned room with a 12 h light-12 h dark cycle. They were given food (Oriental MF, Oriental Yeast Co., Tokyo) and water *ad libitum*. Animals were divided into 4 groups. Group 1: Thirty mice were given 0.5 mg of MNU per mouse once a week for a total of 10 times by intragastric intubation and 1 week after the last MNU administration, the forestomach was resected by operation. Group 2: Thirty mice were given MNU 0.5 mg/mouse as in group 1 without the forestomach resection. Group 3: Ten mice underwent resection of their forestomachs by operation at the same time as group 1. Group 4: Ten mice were used as non-treated controls. Animals were killed at weeks 20, 30 and 40 in groups 1 and 2 and at week 40 in groups 3 and 4. Excised stomachs were fixed in sublimed formaldehyde or ice-cold acetone and cut into about 6 strips that were embedded in paraffin. Other tissues were carefully checked for macroscopic changes, and tumors and related lesions were similarly fixed in 10% formalin or ice-cold acetone and embedded in paraffin. Tissues were stained with hematoxylin and eosin (H.E.) and by the paradoxical concanavalin A (Con A) method.[9]

The mean body weights of the mice treated with MNU (groups 1 and 2) were significantly lower ($P<0.001$) than those of controls from week 2 to the end of the experiment. However, differences between values for groups 1 and 2 were not significant. The incidences of gastrointestinal tumors observed in each group are summarized in Table I. Tumors developed almost solely

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[2] Abbreviations: MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; MNU, N-methyl-N-nitrosourea; Con A, concanavalin A; H.E., hematoxylin and eosin.

Table I. Sequential Changes in Incidences of Cancers in the Forestomach, Glandular Stomach and Duodenum of Mice Treated with MNU

Weeks & Groups	Effective No. of mice	Forestomach (%)	Glandular stomach[a] (%)				Duodenum (%)
		Squamous cell carcinoma	Well	Poorly	Signet ring	Total tumor-bearing	Well differentiated adenocarcinoma
20 weeks							
MNU+Ope[b]	10	3* (30)	1 [1][c] (10.0)	0	0	1 (10.0)	0
MNU	9	7 (77.8)	1 (11.1)	0	1 (11.1)	1 (11.1)	1 (11.1)
30 weeks							
MNU+Ope	9	3 (33.3)	8[d] [5] (88.9)	2 (22.2)	3 (33.3)	8 (88.9)	3 (33.3)
MNU	5	4 (80.0)	4 [3] (80.0)	0	0	4 (80.0)	0
40 weeks							
MNU+Ope	9	2** (22.2)	9 [2] (100)	1 (11.1)	0	9 (100)	1 (11.1)
MNU	4	4 (100)	4[d] [1] (100)	1[e] (25.0)	1 (25.0)	4 (100)	0
Ope	9	0	0	0	0	0	0
Non-treated	10	0	0	0	0	0	0

a) Well, well differentiated adenocarcinoma; Poorly, poorly differentiated adenocarcinoma; Signet ring, signet ring cell carcinoma.
b) Ope, Forestomach resection.
c) Numbers in square brackets indicate number of intramucosal cancers.
d) One metastasized to lymph nodes.
e) One invaded the pancreas.
*Significantly different from MNU-treated group at $P < 0.05$.
**Significantly different from MNU-treated group at $P < 0.01$.

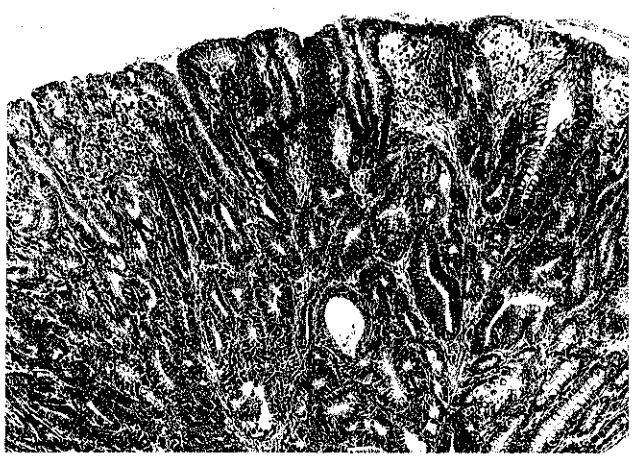

Fig. 1. Adenomatous hyperplasia in group 1 at week 30. H.E., ×100.

in the forestomach, glandular stomach and duodenum. Forestomach lesions were usually large multiple papillary and hyperkeratotic masses occupying the lumen. All tumors of the forestomach were of squamous cell origin. Animals in group 1 which underwent resection also demonstrated squamous cell carcinomas in the remnant squamous epithelium, although the incidence of carcinomas was significantly lower in group 1 ($P < 0.05$–0.01) than in group 2. In addition to those killed at weeks 20, 30 and 40, 2 animals in group 1 and 12 animals in group 2 died spontaneously during the experiment. The small number of effective animals in group 2 was due to a high mortality rate from squamous cell carcinoma development. Most tumors of the glandular stomach were found in the lesser curvature of the pyloric region. Many of them presented as ulcerative nodules with elevated borders, and occasionally marked thickening of the wall of the stomach due to diffuse infiltration of cancer cells was observed. Microscopically, adenomatous hyperplasia (Fig. 1) consisting of excessive glandular proliferation with little or no cellular atypia was found in groups 1 and 2 from 20 weeks at high incidence (all over 75%), increasing with time. At 40 weeks, adenomatous hyperplasias were found in all MNU-treated mice. Carcinomas of the glandular stomach were classified into well-differentiated adenocarcinomas, poorly differentiated adenocarcinomas and signet ring cell carcinomas. The well-differentiated adenocarcinomas were further divided into intramucosal and invasive subtypes. As intramucosal carcinomas (Fig. 2) demonstrated severe cellular atypia, they could be clearly distinguished from the more homogeneous adenomatous hyperplasias. Two well-differentiated adenocarcinomas (one group 1 lesion at week 30 and one group 2 lesion at week 40) showed metastases to regional lymph nodes near the stomach (Fig. 3). Four poorly differentiated adenocarcinomas characterized by reduced tendency for formation of glan-

dular structures and severe cellular atypia were found among 46 effective animals including both groups 1 and 2 at both time points investigated. One of these in a group 2 animal at week 40 invaded the pancreas. Signet ring cell carcinomas were also already found at week 20 in group 2, a total of 5 such lesions (Fig. 4A) developing in total. As more than one type of adenocarcinoma was occasionally found in the same stomach, numbers of tumor-bearing animals for the different categories are indicated in Table I. All cancers originating in the glandular stomach contained tumor cells expressing Class III mucins distinguishable by paradoxical Con A staining (Fig. 4B).

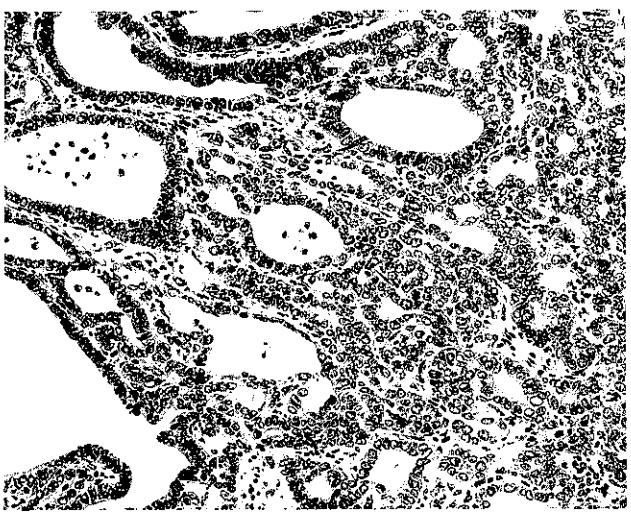

Fig. 2. Intramucosal well differentiated adenocarcinoma in group 1 at week 40. H.E., ×200.

Fig. 3. Metastatic well differentiated adenocarcinoma in a regional lymph node of the stomach in group 2 at week 40. H.E., ×200.

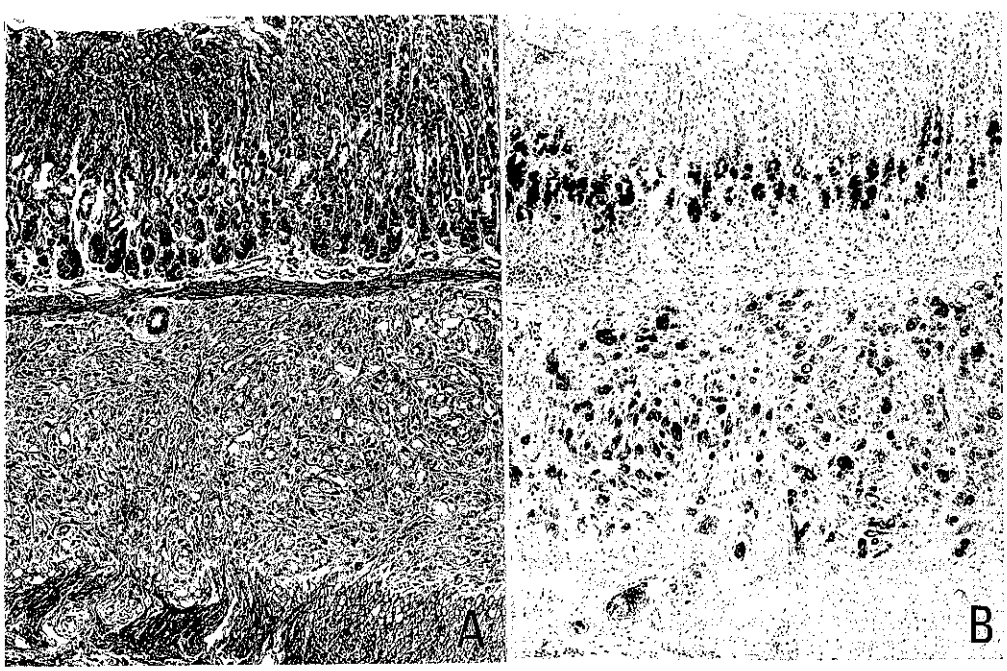

Fig. 4. A: Signet ring cell carcinoma in group 1 at week 30. H.E., ×300. B: Serial section of the same specimen as in Fig. 4A consisting of class III mucin-positive cells; paradoxical Con A staining, ×300.

Intestinal metaplasias were not found in the stomachs of any of the mice in this experiment. Well-differentiated adenocarcinomas of the duodenum were present in 5 of 46 MNU-treated effective animals. One hemangioma of the spleen was also found in an MNU-treated animal in group 1 at week 40. In control animals of groups 3 and 4, no tumors were found.

Human gastric cancers have been classified into differentiated (papillary and tubular adenocarcinomas) and undifferentiated (poorly differentiated adenocarcinomas and signet ring cell carcinomas) types.[10] In man, both demonstrate local invasion and metastasis to lymph nodes or to other organs. Therefore, in addition to providing a high cancer yield and good survival rates, the optimal experimental animal model for human gastric cancers should also feature both differentiated and undifferentiated types with metastasis. Adenocarcinomas induced by MNNG in the rat stomach are mainly of differentiated type[1-3] and there have been no reports of lymph node metastasis by such lesions in the rat. In the present experiment, in contrast, 2 out of 27 differentiated adenocarcinomas involved regional lymph node metastasis. Even with additional surfactant,[11] salt,[12] gastrin or serotonin[13] in combination with MNNG treatment, the induced incidences of undifferentiated type lesions have remained low. In the present model, simple administration of MNU to mice caused 4 poorly differentiated adenocarcinomas and 5 signet-ring cell carcinomas in 27 glandular stomach tumor-bearing animals. A further advantage was the severe cellular atypia of tumor cells allowing relatively easy distinction of intramucosal adenocarcinomas from adenomatous hyperplasias. Therefore this model might be particularly useful for analysis of early gastric carcinogenesis. It is also of interest that no intestinal metaplasias were noted in the present experiment and all cancers contained class III mucins-positive cells, indicating pyloric gland cell type.[14] Similarly no clear relationship was found between intestinal metaplasia and MNNG-induced glandular stomach cancers in the rat.[14]

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