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Effects of Weaning by Surrogate Mothers (ACI) on Tumor Development in SD Rats Treated with Methylnitrosourea (MNU) and/or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)

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ABSTRACT

In this experiment, methylnitrosourea (MNU) was administered, followed by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), to assess effects of surrogate mothering on tumor. One or two day old male SD pups were treated with or without 30 mg/kg body weight of MNU and nursed by SD or ACI surrogate mothers for 5 weeks. When 6-weeks-old they were then treated with 100 ppm MNNG or tap water for 16 weeks. The tumor incidence in the MNNG alone group was significantly lower than with MNU alone or MNU+MNNG ($p<0.01$). Kidney or nerve tumors mainly developed in the MNU group, gastric tumors in the MNNG group, and the two combined in the MNU+MNNG group. The incidence and mean number of tumors did not significantly differ between the two weaning groups. However, mean survival time with the ACI surrogate mothers after treatment with MNU was increased as compared with the SD mother group. Cumulative development of tumors in the ACI surrogate mother group was also delayed ($p<0.05$). Similar results were obtained with MNU+MNNG and MNNG alone. The present experiment suggested that tumor induction might be effected by components of the mother's milk.

Key words: *Carcinogen, Rat, Mothers milk, Tumor induction*

Regarding factors affecting tumor induction, differences with the species, strain, sex, lot, age at carcinogen treatment and intake of calories are well known. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)[22] is a gastric carcinogen, inducing glandular stomach tumors for example in hamsters[6] and rats[22] and N-methyl-N-nitorosourea (MNU)[4,5] is very strong carcinogen with multiple targets. Female rats are generally less susceptible to the carcinogenicity of MNNG than their male counterparts[16] and castration or injection of estradiol in male rats reduces the incidence of gastric adenocarcinomas induced by MNNG[7,8]. The age at which rats are treated with MNNG is also an important factor determining susceptibility[12,28], tumor yields being greater in young animals. There are, furthermore, remarkable strain differences in susceptibility to MNNG in rats[17,28]. Treatment with MNNG in susceptible ACI rats, resistant Buffalo rats, and their F_1 and F_2 offspring demonstrated that this is genetically determined[17]. A single intragastric dose of MNNG given to Wistar rats produces a high incidence of forestomach tumors but only a few adenocarcinomas in glandular stomach[29]. Mice are generally considered to be resistant to gastric carcinonogenesis[13], but C3H mice given MNU *ad libitum* in drinking water developed adenocarcinomas in glandular stomach[25], like mongrel and beagle dogs given the MNNG treatment[23]. Production of stomach cancer was not achieved with administration of MNNG to rabbits but many carcinomas were observed in the trachea[21]. Lung tumors were induced in 5-day old W/Fu but not in ACI rats given a single intragastric dose of MNNG[28]. Gastric adenocarcinoma in F344 rats exposed to MNU in their drinking water and their animals developed simultaneously with a high incidence of

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tumors in the brain and spinal cord[14].

Thus, there are many factors underlying susceptibility of chemical induced tumor. However, there have been no reports of effects of mother's milk except with regard to virus infection. It is therefore of interest to determine the effects of nursing by other strain mothers on tumor induction. In this experiment MNU was first administered, then MNNG after the weaning period.

MATERIALS AND METHODS

Nine week-old Crj: CD(SD) and ACI/NHos rats were mated for 1 week. SD male rat offspring were used in this experiment. MNU (30 mg/kg) or saline was injected i.p. 1 or 2 days after birth. Seventy five pups were nursed by different SD and fifty by ACI mothers. One litter was separately nursed by 2 ACI mothers for five weeks. When 6-weeks-old the animals were given MNNG 100 mg/liter in light-opaque bottles as their drinking water for 16 weeks. The MNNG solution was exchanged at 2- to 3-day intervals. Separate groups were included receiving MNU or MNNG alone. The rats were maintained under guidelines set forth in the Guide for the Care and Use of Laboratory Animals established by Hiroshima University. All were fed a normal MF diet (Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water *ad libitum*.

They were autopsied when they became moribund at the final sacrifice 380 days after the initial MNNG treatment. An autopsy was performed under ether anesthesia at which time the body and major organ weights were measured. The excised tissues were fixed and sections were routinely prepared for histological evaluation.

Statistical significance was determined with the Dunnett method for multiple comparisons, Cox proportional hazard model for comparison logarithmic transformation, and the X^2 test.

RESULTS

The first tumor appeared at 45 days in the MNU group and all rats which survived until this time point were therefore included in effective numbers. Mean survival in the MNU-treated groups was significantly shorter than in the MNU+MNNG and MNNG groups by Dunnett test (Table 1), and in the group with SD mothers was shorter than with ACI mothers in the MNU-treated group ($p<0.05$). There were no significant differences in the MNU+MNNG and MNNG groups.

Body weights in the MNNG group were heaviest, followed by the MNU+MNNG, then the MNU cases. Body weights in SD mother-nursed animals were significantly greater than with ACI mothers, in the MNNG group, between 30 to 120 days of first MNNG treatment, and in the MNU group between 60 and 90 days (Fig. 1).

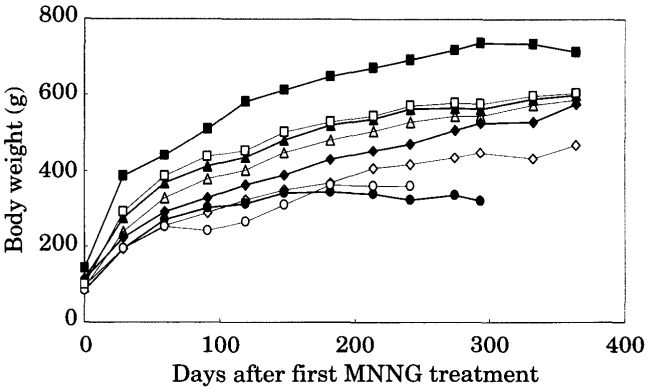

Fig. 1. Body weight curve

—●— MNNG+MNU SD; —○— MNNG+MNU ACI
—▲— MNNG SD; —△— MNNG ACI
—◆— MNU SD; —◇— MNU ACI
—■— Control SD; —□— Control ACI

Table 1. Mean survival and tumor induction

Group	Mean survival	Effective animals	Tumor bearing animals	Mean number of tumors
MNU+MNNG (SD)	293.0 ± 87.7	30	27 (90)	1.63 ± 0.89
MNU+MNNG (ACI)	302.9 ± 69.0	11	8 (67)	1.00 ± 0.95
MNU+MNNG (Total)	295.7 ± 82.4[a,b]	41	35 (85)[b]	1.49 ± 0.93[b]
MNNG (SD)	378.3 ± 23.3	24	9 (34)	0.42 ± 0.64
MNNG (ACI)	376.4 ± 12.4	12	4 (33)	0.33 ± 0.49
MNNG (Total)	377.7 ± 20.3[a,c]	36	13 (36)[b,c]	0.39 ± 0.59[b,c]
MNU (SD)	130.4 ± 51.2*	5	4 (80)	1.60 ± 1.34
MNU (ACI)	217.3 ± 73.2*	12	11 (92)	2.00 ± 1.41
MNU (Total)	191.7 ± 77.5[b,c]	17	15 (88)[c]	1.88 ± 1.36[c]
Control (ACI)	381	16	0	0
Control (SD)	380	15	0	0

For pairs ;a, $p < 0.01$; b c, *, $p < 0.05$ (Mean survival and mean number of tumors by used Dunnett test, Tumor bearing animals by used X^2 test)

Table 2. Body, organ and relative weights

Group	Effective no of animals	Body (g)	Liver (g)	Kidney (g)	Testis (g)	Adrenal (g)	Spleen (g)
MNU+MNNG (SD)	30	454 ± 132	12.7 ± 4.0 (28.1 ± 4.6)	9.3 ± 20.5 (2.30 ± 5.18)	1.36 ± 0.93 (0.29 ± 0.19)	0.048 ± 0.017 (0.011 ± 0.004)	0.89 ± 0.39 (0.20 ± 0.08)
MNU+MNNG (ACI)	11	395 ± 94	11.6 ± 3.7 (29.1 ± 6.5)	9.5 ± 16.5 (2.49 ± 4.16)	1.49 ± 1.01 (0.37 ± 0.24)	0.068 ± 0.081 (0.018 ± 0.022)	1.03 ± 0.44 (0.27 ± 0.12)
MNU+MNNG (Total)	41	438 ± 125[a,d]	12.4 ± 3.9[a,d] (28.4 ± 5.1[e,f])	9.3 ± 19.2 [a,c] (2.35 ± 4.86[e])	1.40 ± 0.94 (0.31 ± 0.20[e,f])	0.054 ± 0.045 (0.013 ± 0.012)	0.93 ± 0.40[a] (0.22 ± 0.10[e])
MNNG (SD)	26	577 ± 102	14.6 ± 2.9 (25.3 ± 2.9)	3.1 ± 0.5 (0.55 ± 0.07)	3.35 ± 0.46[a] (0.59 ± 0.09)	0.062 ± 0.015 (0.011 ± 0.003)	0.81 ± 0.20 (0.14 ± 0.04)
MNNG (ACI)	12	587 ± 65	15.2 ± 1.8 (26.0 ± 1.1)	2.8 ± 0.4 (0.49 ± 0.05)	2.93 ± 0.22[a] (0.50 ± 0.04)	0.055 ± 0.010 (0.009 ± 0.002)	0.83 ± 0.20 (0.14 ± 0.04)
MNNG (Total)	38	580 ± 91[a,b]	14.8 ± 2.6[a,d] (25.5 ± 2.5[e,g])	3.1 ± 0.4 [a,b] (0.53 ± 0.07[e])	3.21 ± 0.44 (0.56 ± 0.09[e,g])	0.060 ± 0.014 (0.011 ± 0.003)	0.82 ± 0.19[d] (0.14 ± 0.04[e,f])
MNU (SD)	5	245 ± 75	9.0 ± 4.5 (35.3 ± 8.4)	2.9 ± 1.7 (1.19 ± 0.74[f])	0.46 ± 0.09 (0.20 ± 0.07[e])	0.039 ± 0.012 (0.018 ± 0.010)	0.56 ± 0.19 (0.31 ± 0.12)
MNU (ACI)	12	305 ± 72	10.2 ± 2.5 (33.8 ± 5.4)	12.9 ± 23.0 (3.90 ± 6.57[f])	0.41 ± 0.25 (0.12 ± 0.07[e])	0.075 ± 0.126 (0.025 ± 0.041)	0.81 ± 0.48 (0.19 ± 0.06)
MNU (Total)	17	287 ± 76[b,d]	9.8 ± 3.1[b,d] (34.3 ± 6.2[e,g])	10.0 ± 20.0 [b,c] (3.11 ± 5.60)	0.42 ± 0.21 (0.15 ± 0.07[f,g])	0.064 ± 0.106 (0.023 ± 0.035)	0.64 ± 0.31[a,d] (0.23 ± 0.09[f]),
Control (ACI)	16	598 ± 67	15.8 ± 2.5 (26.4 ± 3.0)	3.1 ± 0.3 (0.53 ± 0.07)	2.89 ± 0.22 (0.49 ± 0.06)	0.064 ± 0.031 (0.011 ± 0.006)	0.77 ± 0.08 (0.13 ± 0.02)
Control (SD)	15	611 ± 32	17.6 ± 0.9 (28.8 ± 14.7)	3.1 ± 0.4 (0.51 ± 0.05)	3.41 ± 0.24 (0.54 ± 0.19)	0.063 ± 0.001 (0.011 ± 0.020)	0.77 ± 0.09 (0.13 ± 0.05)

( ); Relative weight
For pairs: a, b, c, f, g, $p < 0.01$; d, e, $p < 0.05$ by used Dunnett test

Table 3. Tumor induction (%)

Group	Effective animals	Glandular stomach	Duodenum	Kidney	Brain	Spinal cord	Apocrine	Nerve	Papilloma+ Squamous cell carcinoma	Sarcoma	Other
MNU+MNNG (SD)	30	8 (27)	5 (17)	9 (30)	3 (10)	6 (20)	3 (10)	2 (7)	5 (17)	2 (7)	6 (20)
MNU+MNNG (ACI)	11	2 (18)	0	1 (9)	0	0	0	1 (9)	2 (18)	1 (9)	1 (9)
MNU+MNNG (Total)	41	10 (24)	5 (12)	10 (24)	3 (7)	6 (15)	3 (7)	3 (7)	7 (17)	3 (7)	7 (17)
MNNG (SD)	24	7 (29)	3 (13)	0	0	0	0	0	0	1 (4)	0
MNNG (ACI)	12	2 (17)	1 (8)	0	0	0	0	0	1 (8)	0	0
MNNG (Total)	36	9 (25)	4 (11)	0	0	0	0	0	1 (3)	1 (3)	0
MNU (SD)	5	0	0	2 (40)	0	2 (40)	1 (10)	0	0	0	1 (10)
MNU (ACI)	12	1 (8)	1 (8)	3 (25)	1 (8)	2 (17)	2 (17)	0	3 (25)	4 (33)	3 (25)
MNU (Total)	17	1 (7)	1 (7)	5 (29)	1 (6)	4 (24)	3 (18)	0	3 (18)	4 (24)	4 (24)
Control (ACI)	16	0	0	0	0	0	0	0	0	0	0
Control (SD)	15	0	0	0	0	0	0	0	0	0	0

Body and liver weights in the MNU groups were significantly lower than the MNU+MNNG and MNNG groups (Table 2). Kidney and testes weights in the MNNG group were lower than in the MNU+MNNG- and MNU-treated cases and testes in the SD mother MNNG group were heavier than the ACI cases.

Tumor bearing animals and mean number of tumors were highest in the MNU group, followed by the MNU+MNNG and then the MNNG alone cases (Table 1). Table 3 summarizes data for tumor induction in various organs. Gastric tumors predominated in the MNNG-treated group while MNU induced various types of lesions, including kidney and nerve tumors. With MNU+MNNG all appeared. The incidence of gastrointestinal tumors in the MNU alone group was lower than in the MNU+MNNG and MNNG alone groups. Lesions arose first in the MNU and then in the MNU+MNNG and finally in the MNNG groups.

The total tumor incidence in all groups with SD mothers was greater than with ACI mothers, at least in the early phase (Fig. 2). Tumor bearing animals, mean number of tumors and types of

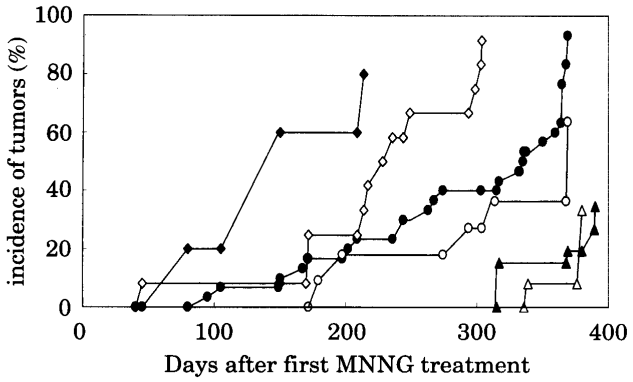

Fig. 2. Incidence of tumor in groups of MNNG+MNU, MNNG and MNU

—●— MNNG+MNU SD; —○— MNNG+MNU ACI
—▲— MNNG SD; —△— MNNG ACI
—◆— MNU SD; —◇— MNU ACI

tumors did not significantly differ with the weaning mother strain for each chemical group. However, tumor development in the group with SD mothers was significantly earlier than with ACI mothers in the MNU and MNU+MNNG groups ($p<0.05$, Fig. 2).

DISCUSSION

The present study showed that tumors developed earlier with SD mothers than with weaning by ACI mothers, this being in line with the body weights. In general, SD rats are heavier than their counterparts of the ACI strain, these being generally considered due to genetic factors although there are no data on milk secreted. It is well established that dietary restriction decreases tumor incidence[9,24,26], and it may be that variation in milk intake was responsible for the differences in tumor induction observed. Further studies are warranted to elucidate milk intake by pups from mothers.

Transmission of an agent responsible for murine mammary tumors in mice in milk during the period of lactation was described by Bittner in 1939[3]. Several mouse leukemia viruses have been found and their propagation by vertical transmission in both mice[10,18,19,27], and rats is an established fact[2,19,20]. Miyamoto et al[15] reported that intraperitoneal injection of serum from colon cancer carrying WF rats induced colon carcinomas in the ascending colon of LE and Wistar/Shi rats when administered during the suckling period, suggesting the existence of some transmissible agent. It is unlikely that any virus was involved in the differences observed here but the results do point to the necessity for looking at factors in mothers milk for understanding of the development of tumors in rats.

Mean survival time in the MNU group was here found to be significantly shorter as compared with in the MNU+MNNG or MNNG groups. Ito et al reported that combined treatment with MNU, DMH and MNNG induced tumors in various sites[11]. In the present case, it was suggested that subsequent MNNG exposure might decrease MNU cancer induction. However, gastric tumorigenesis by MNU+MNNG was similar to that with MNNG alone. During MNNG treatment body weight did not increase to the same extent as in the groups without this carcinogen and this might have inhibited growth of lesions induced by MNU.

In conclusion, the present results are of interest in indicating the importance of the weaning period for development of tumors.

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