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Enhancing Effects of Estrogens on Endometrial Carcinogenesis Initiated by N-Methyl-N-nitrosourea in ICR Mice

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The present study was undertaken to examine the effects of estrogens, such as estrone (E_1), 17β-estradiol (E_2) and estriol (E_3), on endometrial carcinogenesis initiated by N-methyl-N-nitrosourea (MNU) in mice. A total of 120 female ICR mice received MNU solution (1 mg/100 g body wt.) and normal saline at 10 weeks of age into their left and right uterine corpora, respectively. One week later, they were divided into four groups and treated as follows: Group 1 (30 mice) was given 25 ppm E_1-containing diet; Group 2 (30 mice) was fed 5 ppm E_2-containing diet; Group 3 (30 mice) was given 25 ppm E_3-containing diet; and Group 4 (30 mice) was fed the basal diet alone. At the termination of the experiment (Week 30), all surviving animals were autopsied and histopathological examinations revealed that endometrial adenocarcinomas had developed in all groups. The incidence of adenocarcinomas in the MNU-treated uterine corpus in Group 1 (25 ppm E_1-feeding, 9/23, 39%) was significantly higher than that in Group 4 (basal diet, 3/26, 12%, $P<0.05$). Also, the incidences of adenocarcinomas in the MNU-treated uterine corpus in Groups 2 (5 ppm E_2-feeding, 8/24, 33%) and 3 (25 ppm E_3-feeding, 7/26, 28%) were higher than in Group 4, but the difference was not statistically significant. Feeding of diet containing E_1, E_2 and E_3 increased the incidences of the preneoplastic endometrial lesions (atypical, adenomatous or cystic glandular hyperplasia). In the uterine cervix, small numbers of squmous cell carcinomas, dysplasias or hyperplasias were occasionally found in all groups. These results indicate enhancing effects of the above three types of estrogens on the endometrial carcinogenesis induced by MNU in ICR mice.

Key words: Endometrial carcinogenesis — Estrogen — N-Methyl-N-nitrosourea — ICR mouse

The role of estrogens in the etiology of endometrial carcinoma has received considerable research attention. A connection between unopposed estrogen and endometrial cancer has been suggested.[1)] Differential effects of estrone (E_1) or 17β-estradiol (E_2) and estriol (E_3), among natural estrogens, on endometrial carcinogenesis have been reported.[2)] E_1 and E_2 are considered to promote the growth of endometrial cells, while E_3 has been reported to act selectively on vaginal and uterine cervical cells without causing endometrial proliferation.[3)] However, the effects of these three estrogens on endometrial carcinogenesis have remained to be differentially elucidated. Recently, we have developed a rapid induction model of endometrial carcinogenesis in mice and found a synergistic effect of N-methyl-N-nitrosourea (MNU) and E_2.[4)] In the present study, we examined the effect of E_1 or E_3 on endometrial carcinogenesis initiated by MNU, using a modification of the above model, compared with the effect of E_2.

MATERIALS AND METHODS

Animals and chemicals A total of 120 female ICR mice, 10 weeks of age, were purchased from Japan SLC Co. (Shizuoka). Five or six animals were housed in a plastic cage with hardwood chips and kept in an air-conditioned animal room at 25±2°C and 55±5% humidity under a 12-h light/12-h dark cycle during the experiment. The basal diet (Oriental MF, Oriental Yeast Co., Tokyo) and water were available *ad libitum* throughout the experiment.

MNU was purchased from Nacalai Tesque Inc., Kyoto. E_1, E_2 and E_3 were obtained from Sigma Chemical Co. (St. Louis, MO).

Treatment All mice received laparotomy under general anesthesia with diethylether and were injected with MNU solution (total volume: 0.1 ml) using a disposable syringe (23 gauge) at a dose of 1 mg/100 g body wt. to the left uterine tube and normal saline to the right uterine tube. One week after the exposure to MNU, the animals were divided into four experimental groups. Groups 1–3 were given diet containing a natural estrogen, E_1, E_2 or E_3, respectively. The doses of estrogens were decided as follows: the diet containing 5 ppm E_2 was used in the previous report,[4)] and the doses of E_1 and E_3 were set at five times that of E_2 mainly on the basis of their half-life[5,6)] to give almost the same biological potency on the endometrium as obtained with E_2. Group 1 (30 mice) was fed the diet containing 25 ppm E_1. Group 2 (30 mice) or Group 3 (30 mice) was fed the diet containing

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5 ppm E_2 or 25 ppm E_3, respectively. Group 4 (30 mice) was fed the basal diet alone. The experiment was terminated 30 weeks after the start of the study. At the termination of the experiment, all animals were killed and autopsied. All major organs, especially the reproductive organs, were weighed and carefully inspected grossly. The uterus, ovaries, vagina and other lesions suspected of being neoplastic or hyperplastic were submitted to histological examination. Tissues were processed for histology by the conventional method, and sections (3 μm in thickness) were stained with hematoxylin and eosin (H-E).

Histology of the uterine lesions According to the WHO criteria,[7] uterine endometrial lesions were divided into four groups: cystic glandular hyperplasia, adenomatous hyperplasia, atypical hyperplasia and adenocarcinoma. Uterine cervical lesions were divided into three groups according to the criteria of Muñoz *et al.*[8]: hyperplasia, dysplasia and squamous cell carcinoma.

Statistical analysis Statistical analysis was done by using the χ^2 test or Student's t test.

RESULTS

Seven mice in Group 1, six in Group 2, five in Group 3 and four in Group 4 died within 15 weeks, though no pathological abnormalities other than pneumonia were found. The remaining animals survived until the termination of the experiment and were counted as effective animals.

The mean body weights, and mean weights of the left or right uterine corpus and cervix are summarized in Table I. The mean weight of the left uterine corpus was significantly greater than that of the right uterine corpus in each group ($P<0.05$). However, as regards the mean body weight, mean weight of the left or right uterine corpus, and mean weight of the uterine cervix, no significant differences were found among the four experimental groups.

The gross appearance of the cut surface of the uterus is shown in Fig. 1. The uterine corpora in Groups 1–3 were generally swollen and sometimes contained tumors, particularly in the left uterine corpus. The cut surface of the tumor was whitish-gray in color and compact.

Histological examination revealed neoplasms in the bilateral uterine corpora in all groups. In the uterine corpus, these tumors were well or moderately differentiated adenocarcinomas (Fig. 2). The incidences of the preneoplastic and neoplastic lesions in the uterine corpus are summarized in Table II. The incidence of adenocarcinoma of the left uterine corpus in Group 1 (9/23, 39%) was significantly higher than that in Group 4 (3/26, 12%, $P<0.05$). The incidences of adenocarcinoma

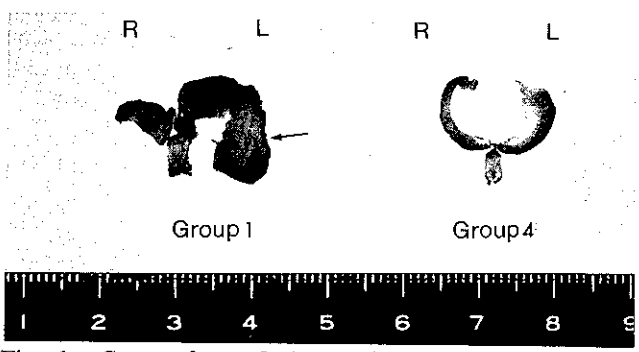

Fig. 1. Cut surface of the uteri of mice in Groups 1 (MNU/saline+E_1) and 4 (MNU/saline alone). The uterine corpora of the mouse in Group 1 are markedly swollen in comparison with those of the mouse in Group 4. A tumor (arrow) with whitish-gray color is located in the left uterine corpus.

Table I. Mean Body Weights, and Mean Weights of Left and Right Uterine Corpus and Cervix

Group (Treatment)	Initial number of animals	Effective number of animals[a]	Body weight (g)	Wet weight of uterine corpus (g)		Wet weight of uterine cervix (g)
				Left	Right	
Group 1 (MNU/saline+E_1)	30	23	45.5±5.5[b]	0.57±0.50[c]	0.34±0.12	0.26±0.13
Group 2 (MNU/saline+E_2)	30	24	42.8±5.0	0.71±0.25[c]	0.35±0.16	0.30±0.15
Group 3 (MNU/saline+E_3)	30	25	43.1±3.1	0.67±1.51[c]	0.33±0.11	0.36±0.14
Group 4 (MNU/saline alone)	30	26	48.0±5.9	0.81±1.70[c]	0.33±0.20	0.22±0.11

a) Animals that survived more than 15 weeks.
b) Mean±SD.
c) Significantly heavier than the right side ($P<0.05$).

of the left uterine corpus in Groups 2 (8/24, 33%) and 3 (7/26, 28%) tended to be increased over that in Group 4. In the right uterine corpus, small numbers of adenocarcinomas were observed in all groups. Besides the neoplasms, preneoplastic lesions of the uterine corpus were also found. Atypical hyperplasia (Fig. 3) and adenomatous hyperplasia (Fig. 4) were seen in Groups 1–4 (Table II). The incidences of atypical hyperplasia of the left uterine corpus in Groups 1 (14/23, 61%) and 2 (16/24, 67%) were significantly greater than that in Group 3 (8/25, 32%, $P<0.05$) or 4 (7/26, 27%, $P<0.01$). The incidence of adenomatous hyperplasia of the left uterine corpus showed no differences among all the groups. The incidences of cystic glandular hyperplasia of the left uterine corpus in Groups 1 (19/23, 83%), 2 (22/24, 92%) and 3 (24/25, 96%) were significantly higher than that in Group 4 (5/26, 19%, $P<0.01$).

In the right uterine corpus treated with normal saline, hyperplastic lesions were present, but their incidences were generally decreased compared with those found in the left uterine corpus. The incidences of adenomatous hyperplasia of the right uterine corpus in Groups 1 (21/23, 91%) and 2 (23/24, 96%) were significantly higher than that in Group 4 (12/26, 46%, $P<0.001$). The incidences of cystic glandular hyperplasias in the right uterus in Groups 1 (19/23, 83%), 2 (21/24, 88%) and 3 (23/25, 92%) were significantly higher than that in Group 4 (1/26, 4%, $P<0.001$).

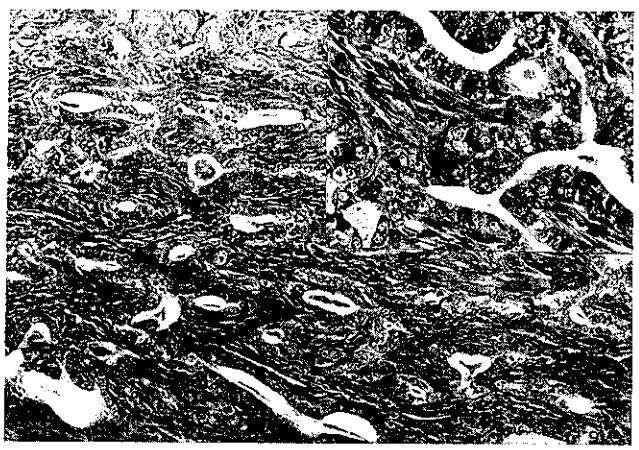

Fig. 2. Moderately differentiated adenocarcinoma of the endometrium in a mouse treated with MNU and E_1. ×70. Inset: High-power view of the same lesion. Mitoses are frequently present in atypical glands. H-E. ×320.

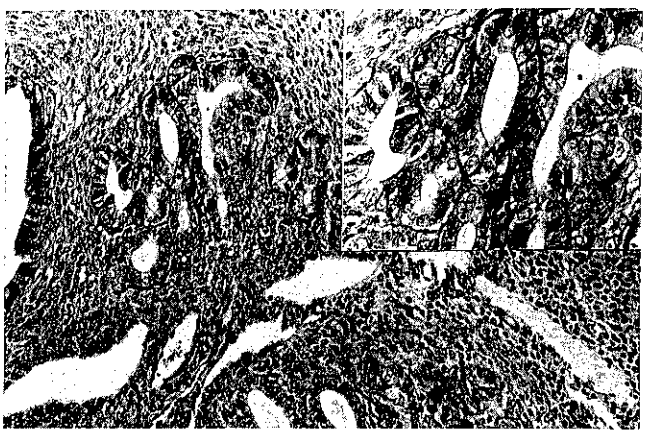

Fig. 3. Atypical hyperplasia of the endometrium in a mouse treated with MNU and E_1. ×180. Inset: "Back-to-back crowding" with slightly cytological atypia is apparent. H-E. ×320.

Table II. Incidence of Preneoplastic and Neoplastic Endometrial Lesions in Each Group

Group (Treatment)	Effective number of animals[a]	Left uterus Number of animals with				Right uterus Number of animals with			
		CGH[b]	AdH	AtH	ADC	CGH	AdH	AtH	ADC
Group 1 (MNU/saline+E_1)	23	19[c] (83%)	22 (96%)	14[d] (61%)	9[d] (39%)	19[c] (83%)	21[c] (91%)	10[e] (43%)	2 (9%)
Group 2 (MNU/saline+E_2)	24	22[c] (92%)	23 (96%)	16[c,f] (67%)	8 (33%)	21[c] (88%)	23[c] (96%)	7 (29%)	4 (8%)
Group 3 (MNU/saline+E_3)	25	24[c] (96%)	23 (92%)	8 (32%)	7 (28%)	23[c] (92%)	14 (52%)	5 (20%)	2 (8%)
Group 4 (MNU/saline alone)	26	5 (19%)	20 (77%)	7 (27%)	3 (12%)	1 (4%)	12 (46%)	2 (8%)	1 (4%)

a) Animals that survived more than 15 weeks.
b) CGH, cystic glandular hyperplasia; AdH, adenomatous hyperplasia; AtH, atypical hyperplasia; ADC, adenocarcinoma.
c–e) Significantly higher than that in Group 4 (*c*, $P<0.001$; *d*, $P<0.05$; *e*, $P<0.01$).
f) Significantly higher than that in Group 3 ($P<0.05$).

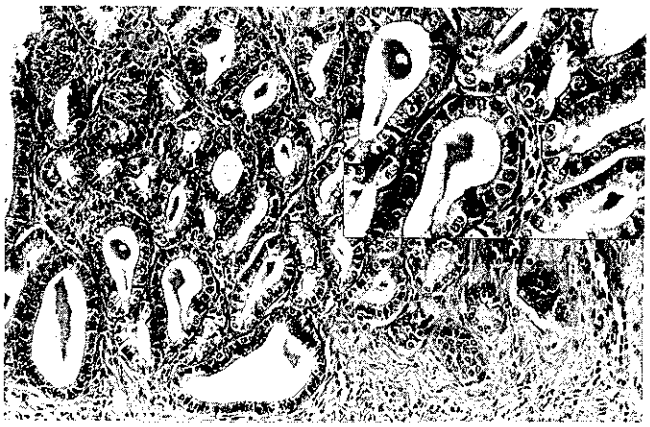

Fig. 4. Adenomatous hyperplasia of the endometrium in a mouse treated with MNU and E_1. ×180. Inset: "Back-to-back crowding" is present in the lesion. However, cytological atypia is absent. H-E. ×320.

Fig. 5. Dysplasia of the uterine cervix in a mouse treated with MNU and E_1. H-E. ×135.

Squamous cell carcinomas, dysplasias (Fig. 5) and hyperplasias of the uterine cervix were found occasionally in Groups 1 to 4, as shown in Table III. In addition, a few non-epithelial tumors were found only in the uterine corpus in Groups 1, 2 and 4. These were histologically leiomyosarcomas and hemangiomas. In other organs, one mammary adenocarcinoma was seen in a mouse in Group 4.

Table III. Incidence of Preneoplastic and Neoplastic Uterine Cervical Lesions in Each Group

Group (Treatment)	Effective number of animals[a]	Number of animals with		
		Hyperplasia	Dysplasia	SCC[b]
Group 1 (MNU/saline+E_1)	23	7[c] (30%)	1 (4%)	0 (0%)
Group 2 (MNU/saline+E_2)	24	8[c] (32%)	2 (8%)	0 (0%)
Group 3 (MNU/saline+E_3)	25	11[c] (44%)	0 (0%)	0 (0%)
Group 4 (MNU/saline alone)	26	1 (4%)	1 (4%)	1 (4%)

a) Animals that survived more than 15 weeks.
b) SCC, squamous cell carcinoma.
c) Significantly higher than that in Group 4 ($P<0.001$).

DISCUSSION

In the present study, the incidence of endometrial adenocarcinoma in the group treated with MNU and 25 ppm E_1-feeding was significantly higher than that in the group treated with MNU alone ($P<0.05$). The incidence of adenocarcinoma in the group treated with MNU and 5 ppm E_2- or 25 ppm E_3-feeding tended to be increased compared with that of the group treated with MNU alone, though the difference was not statistically significant. Although E_1, E_2 and E_3 have enhancing effects on MNU-induced endometrial carcinogenesis, the dose of E_1 needed was 5 times higher than that of E_2 for exerting a similar enhancing effect to E_2.

In animal experiments, hormonal dysregulation, namely an increased estrogen/progesterone ratio has been considered to be associated with endometrial carcinogenesis in mice[4] and rats.[9] The relative carcinogenic potentials of E_1 and E_2 on the endometrium have been discussed.[2] E_2 is biologically potent due to its long half-life (48 h), but is readily oxidized to E_1. E_1 is considered to be intermediate in biological potency (half-life: 24 h).[5,6] Thus, in the present study, E_1 was used at five times the concentration (25 ppm) of E_2 (5 ppm), and this is the first report demonstrating an enhancing effect of E_1 on MNU-induced mouse endometrial carcinogenesis. These results are consistent with previous reports that E_1 was exclusively produced in patients with endometrial cancer[1] and that postmenopausal women possessed a relatively high concentration of E_1.[10]

Previously, we have shown that the incidence of endometrial adenocarcinoma was 23% in a group treated with E_2 (5 ppm) diet for twenty weeks.[4] Thus, the carcinogenicity of continuous feeding of E_2 on the endometrium in mice was apparent. In the present study, we sought to examine the effects of other natural estrogens, E_1 and E_3, under the same hormonal condition, for comparison with the effect of E_2. Therefore, MNU solution was injected into the left uterine corpus, while normal saline was injected into the right uterine corpus.

A small amount of the MNU solution might flow to the other uterine corpus, but most of the solution was considered to remain on the side into which it was injected. This is suggested by the different incidence of the tumor development between the left and right uterine corpus. Therefore, it is considered that the right uterus given saline alone in mice fed E_1, E_2 and E_3 might be a valid control. In addition, the weight of the left uterine corpus was significantly heavier than the right uterine corpus in each group. The difference was considered to be due to the presence of adenocarcinoma and other preneoplastic lesions.

It has been reported that E_3, an end product of E_1 and E_2, given by various routes of administration, appears to have a selective proliferative activity in the epithelium of the uterine cervix and vagina, but not on the endometrium.[11, 12] Thus, postmenopausal women, suffering from climacteric syndorome, have been treated with E_3. However, a more recent report has shown that oral administration of E_3 caused endometrial hyperplasia in postmenopausal women.[13] This finding is supported by the result that E_3-feeding tended to have an enhancing effect on the occurrence of endometrial hyperplasia during MNU-initiated endometrial carcinogenesis in mice. In the present study, the continuous long-term administration of E_3 with a relatively weak estrogenic potential may have had a proliferative effect on the endometrium in mice. Further studies will be needed to elucidate the relation between E_3 and risk of endometrial carcinogenesis in postmenopausal women.

It is suggested that the three estrogens all have enhancing effects on endometrial carcinogenesis induced by MNU in mice, based on the increased incidences of endometrial adenocarcinomas and preneoplastic lesions.

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