Jpn. J. Cancer Res. 86, 724–729, August 1995

Inhibitory Effects of Medroxyprogesterone Acetate on Mouse Endometrial Carcinogenesis

Kenji Niwa,[1,3] Shigeo Morishita,[1] Toshiko Murase,[1] Naoki Itoh,[1] Takuji Tanaka,[2] Hideki Mori[2] and Teruhiko Tamaya[1]

Departments of [1]Obstetrics & Gynecology and [2]Pathology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500

The present study was undertaken to examine the effects of cyclic administration of low-dose progestogen on endometrial carcinogenesis in mice. A total of 115 female ICR mice, 10 weeks of age, were divided into four experimental and control groups. Mice in groups 1–3 received laparotomy and were injected with N-methyl-N-nitrosourea (MNU) solution at a dose of 1 mg/100 g body weight to the left uterine tube and with normal saline to the right uterine tube. From one week after the MNU exposure, groups 1 and 2 were given 5 ppm 17β-estradiol (E_2)-containing diet throughout the experiment. Mice in group 1 received 5 s.c. injections of medroxyprogesterone acetate (MPA) (2 mg/mouse) at intervals of 4 weeks from week 7. Group 3 was treated with MNU/normal saline alone. Group 4 consisted of mice treated with MPA alone. At the termination of the experiment (week 30), all animals were killed and autopsied for pathological examinations. It was found that adenocarcinomas and preneoplastic lesions developed in the bilateral uterine corpora in mice of groups 1–3. MPA treatment significantly decreased the weight of the uterine corpus ($P<0.05$) and the incidences of endometrial adenocarcinoma and atypical or adenomatous ($P<0.001$) but not cystic glandular hyperplasias in the MNU/E_2-treated groups. Additionally, MPA treatment tended to decrease the proliferating cell nuclear antigen-labeling index in endometrial glandular cells. These data indicate that MPA, even at low dose, has an inhibitory effect on mouse endometrial carcinogenesis induced by MNU and E_2.

Key words: Inhibition — Carcinogenesis — Medroxyprogesterone acetate — Endometrium — Mouse

The growth of endometrial cancer has been considered to be estrogen-dependent for over 50 years.[1] The incidence has been increasing in Japan[2] as well as in Western countries, plausibly due to estrogen-related nutrition. The current view is that the disease might result from an excess of estrogen accompanied with inadequate cyclic exposure to progestogen.[3] Recent evidence suggests that use of estrogen/progestogen combined oral contraceptives,[4,5] and progestogen for estrogen replacement therapy could prevent[6] occurrence of endometrial cancer. In addition, progestogen is considered to reverse preneoplastic or neoplastic lesions of the endometrium.[7,8] Medroxyprogesterone acetate (MPA) has been used extensively in treatments of endocrine disorders and cancers in the breast and endometrium.[9,10] Although MPA has been reported to be a non-genotoxic carcinogen for the breast,[11–13] the effects of MPA on experimental endometrial carcinogenesis have remained to be clarified.

Recently, we have developed a rapid induction model for endometrial carcinogenesis by using N-methyl-N-nitrosourea (MNU) and 17β-estradiol (E_2).[14,15] In the model, MNU is applied in the uterine corpus by a single injection, followed by feeding of a diet containing E_2.[15] Using this model, a number of endometrial adenocarcinoma and preneoplastic endometrial lesions that mimic human ones develop within 30 weeks. The enhancing effects of various types of natural estrogens on endometrial carcinogenesis induced by MNU were demonstrated by modifying the above model,[15] showing that this experimental model is useful to examine the modifying effects of xenobiotics.

The present study was conducted to examine the effect of MPA on mouse endometrial carcinogenesis using this experimental model. This experiment could also be regarded as an examination of the effect of progestogen for estrogen replacement therapy on endometrial carcinogenesis, where MNU was single-injected, E_2 was continuously administered and MPA was given by low-dose and cyclic administration. In addition, the levels of proliferating cell nuclear antigen (PCNA) were measured in histologically normal glandular cells of the endometrium to clarify the effects of MPA.

MATERIALS AND METHODS

Animals and chemicals A total of 115 female ICR mice (Japan SLC Co., Shizuoka), 10 weeks of age, were housed in groups of 5 or 6 animals per plastic cage and

[3] To whom requests for reprints should be addressed.

kept in an air-conditioned animal room at 25±5°C temperature and 55±5% relative humidity, under a 12-h light/12-h dark cycle during the experiment. The basal diet (Oriental MF, Oriental Yeast Co., Tokyo) and distilled water were available *ad libitum* throughout the experiment. MNU was purchased from Nacalai Tesque Inc. (Kyoto) and E_2 was obtained from Sigma Chemical Co. (St. Louis, MO). MPA was kindly provided by Kyowa Hakko Inc. (Tokyo).

Treatment The experimental design is shown in Fig. 1. Mice in groups 1–3 received laparotomy under general anesthesia with diethylether and were injected with MNU solution (total volume: 0.1 ml) using a disposable syringe (26 gauge) at a dose of 1 mg/100 g body weight into the left uterine tube and with normal saline of equal volume into the right uterine tube. One week after the MNU exposure, the animals were divided into three experimental groups. Groups 1 (35 mice) and 2 (30 mice) were given 5 ppm E_2-containing diet throughout the experiment. In addition, mice in group 1 were given five s.c. injections of MPA at a dose of 2 mg/mouse every 4 weeks starting at week 7. After the exposure to MNU/normal saline, group 3 (30 mice) received basal diet alone. Group 4 (20 mice) was given s.c. MPA injections alone. The experiment was terminated 30 weeks after the MNU exposure. At the termination of the experiment, all animals were killed and autopsied. All major organs, especially the reproductive organs, were weighed and grossly inspected. The uterus, ovaries, vagina and other lesions suspected of being neoplastic and 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.

Histology of the uterine lesions According to the WHO criteria,[16] uterine endometrial lesions were divided into four lesions: cystic glandular hyperplasia, adenomatous hyperplasia, atypical hyperplasia and adenocarcinoma. Uterine cervical lesions were basically diagnosed according to the criteria of Muñoz *et al.*[17] and divided into 3 main lesions: hyperplasia, dysplasia and squamous cell carcinoma.

Immunohistochemical staining for PCNA The PCNA-labeling index was evaluated in pathologically normal endometrial glandular cells in several mice of each group by use of the avidin-biotin-peroxidase complex (ABC) method described by Hsu *et al.*[18] After deparaffinization, endometrial sections were treated sequentially with normal goat serum, anti-PCNA antibody (DAKO Japan Co., Ltd., Tokyo), biotin-labeled goat anti-rabbit IgG (1:400) and ABC. The peroxidase binding sites were demonstrated by the diaminobenzidine method. PCNA-positive nuclei were counted under a microscope and expressed as a proportion of over 200 pathologically normal endometrial glandular cells in five mice of each group.

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

RESULTS

Mean body weights and mean weights of the left or right uterine corpus are summarized in Table I. The mean weight of the MNU-treated left uterine corpus was significantly greater than that of the control right uterine corpus in mice of group 2 ($P<0.05$) and was significantly ($P<0.05$) decreased by MPA treatment (group 1). The mean weight of the left or right uterine corpus in

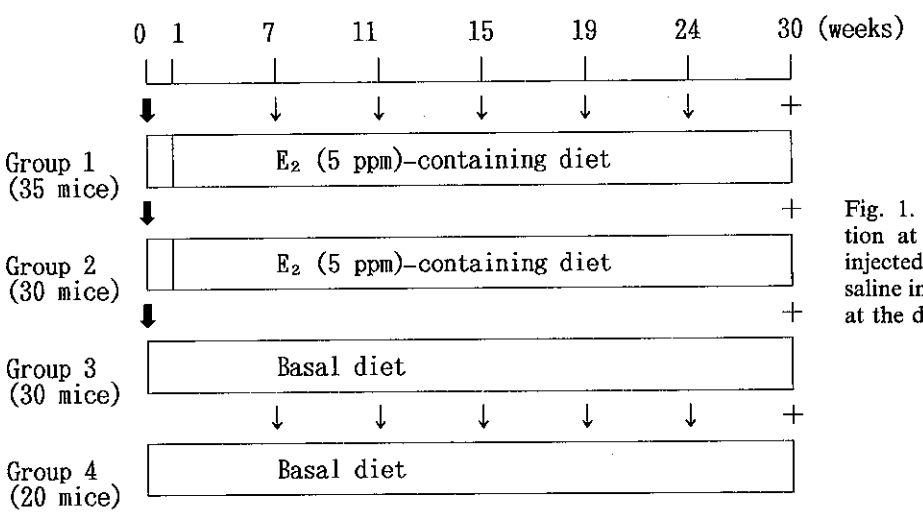

Fig. 1. Experimental design. ⬇: MNU solution at a dose of 1 mg/100 g body wt. was injected into the left uterine tube, and normal saline into the right. MPA (↓) was injected s.c. at the dose of 2 mg/body. E_2, 17β-estradiol.

mice of group 4 was significantly smaller than that in the other three groups ($P<0.001$).

Histological examination revealed that there were neoplasms and preneoplastic lesions in the bilateral uterine corpora in groups 1–3, as shown in Table II. Histologically, these tumors were well or moderately differentiated adenocarcinomas and their occurrences were similar to those in our previous reports.[14, 15] Hardly any difference of histological appearance was found in cases treated with or without MPA. However, slight decidual changes were found in the endometrial stroma in some mice treated with MPA. The incidences of the preneoplastic and neoplastic lesions in the uterine corpus are summarized in Table II. The incidences of adenocarcinoma of the left uterine corpus in mice of group 1 (7%) was significantly lower than that in group 2 (33%) ($P<0.05$). In the right uterine corpus, few adenocarcinomas were observed in mice of groups 1–3.

Besides the neoplasms, preneoplastic lesions of the uterine corpus were also found (Table II). The incidence of atypical hyperplasia in the left uterine corpus of group 1 (13%) was significantly lower than that of group 2 (67%, $P<0.001$). The incidence of adenomatous hyperplasia in mice of group 1 (30%) was significantly lower than that of group 2 (96%, $P<0.001$). However, the incidence of cystic glandular hyperplasia in the left uterine corpus of group 1 (83%) was similar to that of group 2 (92%). In addition, a few non-epithelial tumors were

Table I. Mean Body Weights, and Mean Weights of Left and Right Uterine Corpus of Mice in Each Group

Group (Treatment)	Initial number of animals	Effective number of animals[a]	Body weight (g)	Wet weight of uterine corpus (g)	
				Left	Right
Group 1 (MNU/saline+E_2+MPA)	35	30	42.2±6.5[b]	0.33±0.09[c]	0.26±0.06
Group 2 (MNU/saline+E_2)	30	24	42.8±5.0	0.71±0.25[d]	0.35±0.16
Group 3 (MNU/saline alone)	30	26	48.0±5.9	0.47±0.33[e]	0.33±0.20
Group 4 (MPA alone)	20	20	42.9±3.9	0.07±0.02[f]	0.08±0.03[f]

a) Animals that survived more than 15 weeks.
b) Mean±SD.
c) Significantly smaller than in groups 2 and 3 ($P<0.05$).
d) Significantly heavier than on the right side ($P<0.05$).
e) This value was calculated after exclusion of one animal, which had a large hemangioma in the left uterus.
f) Significantly smaller than in groups 1–3 ($P<0.001$).

Table II. Incidence of Neoplastic and Preneoplastic Lesions in the Mouse Endometrium in Each Group

Group (Treatment)	Effective number of animals[a]	Left uterine corpus Number of animals with				Right uterine corpus Number of animals with			
		CGH[b]	AdH	AtH	ADC	CGH	AdH	AtH	ADC
Group 1 (MNU/saline+E_2+MPA)	30	25 (83%)	9[c,d] (30%)	4[c] (13%)	2[e] (7%)	20 (67%)	10[c] (33%)	4 (13%)	1 (3%)
Group 2 (MNU/saline+E_2)	24	22 (92%)	23 (96%)	16 (67%)	8 (33%)	21 (88%)	23 (96%)	7 (29%)	4 (8%)
Group 3 (MNU/saline alone)	26	5 (19%)	20 (77%)	7 (27%)	3 (12%)	1 (4%)	12 (46%)	2 (8%)	1 (4%)
Group 4 (MPA alone)	20	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)

a) Animals that survived more than 15 weeks.
b) CGH, cystic glandular hyperplasia; AdH, adenomatous hyperplasia; AtH, atypical hyperplasia; ADC, adenocarcinoma.
c–e) Significantly smaller than in group 2 (*c*, $P<0.001$; *e*, $P<0.05$) or group 3 (*d*, $P<0.01$).

Table III. Incidence of Neoplastic and Preneoplastic Lesions in the Uterine Cervix of Mice in Each Group

Group (Treatment)	Effective number of animals[a]	Number of animals with		
		Hyperplasia	Dysplasia	SCC[b]
Group 1 (MNU/saline+E_2+MPA)	30	11[c] (36%)	2 (7%)	0 (0%)
Group 2 (MNU/saline+E_2)	24	8[c] (33%)	2 (8%)	0 (0%)
Group 3 (MNU/saline alone)	26	1 (4%)	1 (4%)	1 (4%)
Group 4 (MPA alone)	20	2 (10%)	0 (0%)	0 (0%)

a) Animals that survived more than 15 weeks.
b) SCC, squamous cell carcinoma.
c) Significantly higher than in group 3 or 4 ($P<0.001$).

Table IV. PCNA-labeling Index of Histologically Normal Glandular Cells in the Endometrium of Each Group

Group (Treatment)	Number of animals examined	PCNA-labeling index
Group 1 (MNU/saline+E_2+MPA)	5	29.3±4.7[a]
Group 2 (MNU/saline+E_2)	5	34.4±16.4
Group 3 (MNU/saline alone)	5	31.4±11.8
Group 4 (MPA alone)	5	27.8±6.2

a) Mean±SD.

found only in the left uterine corpus in mice of groups 2 and 3. These were histologically leiomyosarcomas and hemangiomas.

In the right uterine corpus treated with normal saline, hyperplastic lesions were observed. The incidence of atypical hyperplasia in mice of group 1 (13%) tended to be lower than that of group 2 (29%), but the difference was not statistically significant. The incidence of adenomatous hyperplasia in mice of group 1 (33%) was significantly lower than that of group 2 (96%, $P<0.001$). The incidence of cystic glandular hyperplasia in mice of group 1 (67%) was similar to that of group 2 (88%).

Squamous cell carcinomas, dysplasias and hyperplasias of the uterine cervix were seen in some mice of groups 1–3, as shown in Table III. The incidences of hyperplasia in mice of groups 1 and 2 were significantly higher than those of groups 3 and 4 ($P<0.001$).

In other organs, one mammary adenocarcinoma was found in a mouse of group 3.

Data for PCNA-positive cells per 200 normal glandular cells are shown in Table IV. In histologically normal glandular cells, the PCNA-labeling index in group 1 treated with MPA tended to be smaller than that in group 2, without MPA. However, no significant difference was observed in any group.

DISCUSSION

The results in the present study indicate an inhibitory effect of MPA on endometrial carcinogenesis induced by MNU and E_2 in ICR mice, resulting in reduced incidences of endometrial cancer, atypical hyperplasia and adenomatous hyperplasia in the left uterine corpus (into which MNU solution was directly injected) and a reduced incidence of adenomatous hyperplasia in the right uterine corpus (Table II).

In the right uterine corpus in group 2, the incidences of adenocarcinoma and atypical hyperplasia were relatively low, compared with those in the left uterine corpus, into which the MNU solution was directly injected. This may explain why the inhibitory effect of MPA on the development of these two lesions was not significant in the right uterine corpus (group 1 vs. group 2; Table II).

Long-term E_2 treatment is considered to induce a very high incidence of cystic glandular hyperplasia of the mouse endometrium. MPA treatment at the dose used in the present study failed to reduce significantly the high incidence of cystic glandular hyperplasia in group 2, in contrast to its effect on other preneoplastic and neoplastic endometrial lesions. Cystic glandular hyperplasia may be different from other endometrial lesions.

It has been reported that high dose and/or continuous administration of MPA caused mammary carcinoma or endometrial deciduoma in rodents.[12, 13] In those studies, MPA was used at 40 mg/mouse/two months, while MPA was given at 2 mg/mouse/month in the present study. In the present study, neither deciduoma or mammary carcinoma was seen in the group treated with

MPA, indicating that the dose of MPA used in the present study has no pathogenetic effect on the endometrial stroma or mammary gland. Nagasawa *et al.*[19] reported that MPA inhibits the incidence of precancerous mammary hyperplastic lesions induced by dimethylbenz[*a*]-anthracene. It has been suggested that prolactin is a key hormone for experimental mammary tumorigenesis,[20] and that the low level of prolactin caused by MPA is related to the inhibitory effect of MPA on mammary tumorigenesis.[19] However, the effect of prolactin on endometrial carcinogenesis might not be directly related to the inhibitory effect of MPA in the present study, since prolactin is not related to the growth of the endometrium and its lesions, and MPA was given as cyclic small doses. While the mechanism of MPA inhibition of endometrial carcinogenesis remains to be clarified, progestogen antagonism of endometrial DNA synthesis and nuclear estrogen receptor formation is one possibility.[21, 22]

Cellular proliferation, as reflected by the PCNA-labeling index appears to be related to the action of tumor promoters.[23] In the present study, the PCNA-labeling index in normal endometrial glandular cells tended to be decreased by MPA treatment in MNU/E_2-treated mice. The low level of cell proliferation in MPA-treated mouse might be related to the inhibitory effects of MPA on endometrial carcinogenesis.

The "unopposed-estrogen hypothesis" can account for endometrial cancer risk factors. Certainly the growth of some human endometrial carcinomas depends upon estrogens.[2] In the present study, E_2 was considered to increase the weight of the uterine corpus and to enhance mouse endometrial carcinogenesis, while MPA was thought to decrease the weight and to show an inhibitory effect. Recently, a protective effect of oral contraceptives against endometrial cancer has been reported.[4, 5] Cyclic or continuous administration of progestogen for estrogen replacement therapy decreases the risk of endometrial cancer in human subjects.[6] Thus, it is an interesting possibility that the inhibitory effect of MPA on endometrial carcinogenesis presented here is related to the apparent epidemiological benefits of progestogen supplement in estrogen replacement therapy in humans.

(Received January 23, 1995/Accepted May 17, 1995)

REFERENCES

1) Novak, E. and Yui, E. Relation of endometrial hyperplasia to adenocarcinoma of the uterus. *Am. J. Obstet. Gynecol.*, **32**, 674–679 (1936).
2) Masubuchi, K., Nemoto, H., Masubuchi, S., Fujimoto, I. and Uchino, S. Increasing incidence of endometrial carcinoma in Japan. *Gynecol. Oncol.*, **3**, 335–346 (1975).
3) Ziel, H. K. Estrogen's role in endometrial cancer. *Obstet. Gynecol.*, **60**, 509–515 (1982).
4) Spicer, D. V. and Pike, M. C. The prevention of breast cancer through reduced ovarian steroid exposure. *Acta Oncol.*, **31**, 167–174 (1992).
5) Schlesselman, J. J. Oral contraceptives and neoplasia of the uterine corpus. *Contraception*, **43**, 557–579 (1991).
6) Gambrell, R. D. Prevention of endometrial cancer with progestogens. *Maturitas*, **8**, 159–168 (1986).
7) Gambrell, R. D. The role of hormones in the etiology and prevention of endometrial cancer. *Clin. Obstet. Gynecol.*, **13**, 695–723 (1986).
8) Niwa, K., Yokoyama, Y., Tanaka, T., Murase, T., Morishita, S., Itoh, M., Itoh, N., Shimokawa, K. and Tamaya, T. Successful pregnancy in a patient with endometrial carcinoma treated with medroxyprogesterone acetate. *Arch. Gynecol. Obstet.*, **255**, 91–94 (1994).
9) Lober, J., Mourisden, H. T. and Rose, C. Oral or intramuscular treatment of advanced breast cancer with medroxyprogesterone acetate: a review. *In* "Role of Medroxyprogesterone Acetate in Endocrine Related Tumors. Vol II," ed. L. Campio, G. R. della Cuna and R. W. Taylor, pp. 105–114 (1992). Raven Press, New York.
10) Taylor, R. W. The treatment of endometrial carcinoma with medroxyprogesterone acetate. *In* "Role of Medroxyprogesterone Acetate in Endocrine Related Tumors. Vol. II," ed. L. Campio, G. R. della Cuna and R. W. Taylor, pp. 157–162 (1992). Raven Press, New York.
11) IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 21. "Sex Hormones, Part II," pp. 417–430 (1979). International Agency for Research on Cancer, Lyon.
12) Lanari, C., Molinolo, A. A. and Pasqualini, C. D. Induction of mammary adenocarcinomas by medroxyprogesterone acetate in BALB/c female mice. *Cancer Lett.*, **33**, 215–223 (1986).
13) Molinolo, A. A., Lanari, C., Charreau, E. H., Sanjuan, N. and Pasqualini, C. D. Mouse mammary tumors induced by medroxyprogesterone acetate: immunohistochemistry and hormonal receptors. *J. Natl. Cancer Inst.*, **79**, 1341–1350 (1987).
14) Niwa, K., Tanaka, T., Mori, H., Yokoyama, Y., Furui, T., Mori, H. and Tamaya, T. Rapid induction of endometrial carcinoma in ICR mice treated with N-methyl-N-nitrosourea and 17β-estradiol. *Jpn. J. Cancer Res.*, **82**, 1391–1396 (1991).
15) Niwa, K., Murase, T., Furui, T., Morishita, S., Mori, H., Tanaka, T., Mori, H. and Tamaya, T. Enhancing effects of estrogens on endometrial carcinogenesis initiated by N-methyl-N-nitrosourea in ICR mice. *Jpn. J. Cancer Res.*, **84**, 951–955 (1993).
16) Poulsen, H. E. and Taylor, C. W. Histological typing of

female genital tract tumors. *In* "International Histological Classification of Tumours, No. 13," pp. 63–66 (1975). WHO, Geneva.

17) Muñoz, N., Dunn, T. B. and Turusov, V. S. Tumours of the vagina and uterus. *In* "Pathology of Tumours in Laboratory Animals. Vol II: Tumours of the Mouse," ed. V. S. Turusov, IARC Scientific Publications No. 23, pp. 359–383 (1979). International Agency for Research on Cancer, Lyon.
18) Hsu, S. M., Raine, L. and Fanger, H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.*, **29**, 577–580 (1980).
19) Nagasawa, H., Fujii, M. and Hagiwara, K. Inhibition by medroxyprogesterone acetate of precancerous mammary hyperplastic alveolar nodule formation in mice. *Breast Cancer Res. Treat.*, **5**, 31–36 (1985).
20) Welsch, C. W. and Nagasawa, H. Prolactin and murine mammary tumorigenesis: a review. *Cancer Res.*, **37**, 951–963 (1977).
21) Whitehead, M. I., Townsend, P. T., Pryse-Davies, J., Ryder, T., Lane, G., Siddle, N. C. and King, R. J. Effects of various types and dosages of progestogens on postmenopausal endometrium. *J. Reprod. Med.*, **27**, 539–548 (1982).
22) Whitehead, M. I., Townsend, P. T., Pryse-Davies, J., Ryder, T., Lane, G., Siddle, N. and King, R. J. B. Actions of progestins on morphology and biochemistry of the endometrium of postmenopausal women receiving low-dose estrogen therapy. *Am. J. Obstet. Gynecol.*, **142**, 791–795 (1982).
23) Cohen, S. M. and Ellwein, L. B. Cell proliferation in carcinogenesis. *Science*, **249**, 1007–1011 (1990).