Effect of Progesterone on Cell Division in Chemically Induced Endometrial Hyperplasia and Adenocarcinoma in Mice

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ABSTRACT

Cotton string coated with 3-methylcholanthrene (MCA) was implanted in the uterine cavity of ICR mice to induce endometrial hyperplasia and adenocarcinoma. A low dose (total, 2.5 mg) or a high dose (total, 35 mg) of progesterone was administered to the mice at various times during a period of 4 to 40 weeks after MCA application. After serial labeling with [3H]thymidine, the mice were sacrificed, and thymidine-labeling index values of endometrial hyperplasia and adenocarcinoma both in the progesterone-treated and the untreated mice were investigated by autoradiographic techniques.

In mice that had been implanted with MCA, there was a progressive increase of hyperplasia and neoplasia as a function of time. The low dose of progesterone administered to the mice caused a significant reduction in labeling with [3H]thymidine in nonatypical hyperplasia and moderate atypical hyperplasia, compared to that for the untreated mice. In marked atypical hyperplasia and adenocarcinoma, irrespective of the histological grade, labeling was not reduced.

With the high dose of progesterone, marked morphological alterations with degenerative changes were observed in atypical hyperplasia and differentiated adenocarcinoma. However, cancer cells were still maximally labeled. The results indicate that the effect of progesterone on nonatypical hyperplasia and moderate atypical hyperplasia is mitotic arrest, whereas the effect on marked atypical hyperplasia and adenocarcinoma is morphological and cytological alterations.

INTRODUCTION

The spontaneous development of endometrial adenocarcinoma in experimental animals rarely occurs. The so-called "string method" (18, 21), i.e., the insertion of a cotton string coated with MCA into the uterus, is a reliable technique for production of endometrial hyperplasia and adenocarcinoma in mice. In mice previously implanted with MCA by this method, the hyperplastic stage began in the 4th week (2), and endometrial adenocarcinoma developed by the 20th week of MCA application (22).

It is well documented that the uterine epithelial proliferative response in mice is under the control of steroid hormones (4-6, 10-12). Current investigations have indicated that progesterone blocks uterine epithelial cells in the early G1 phase of the cell cycle (4, 16) and induces these cells to enter the resting (G0) stage (7, 11, 12).

In clinical studies, about one-third of the patients with endometrial carcinoma responded to the progesterational agents with tumor regression (14, 19). Furthermore, it has been suggested that these agents are acting directly at a cellular level (1, 8, 13). However, the exact mechanism of action of these agents in cancer cells has yet to be identified.

In this study the effect of progesterone on MCA-induced endometrial hyperplasia and adenocarcinoma in mice was investigated by autoradiographic techniques after injections of [3H]thymidine. A change in uptake of [3H]thymidine in hyperplasia and neoplasia after treatment with progesterone was statistically evaluated to determine whether or not an early effect of progesterone on these cells was mitotic arrest.

MATERIALS AND METHODS

Animals. Female 8-week-old ICR mice (Nippon Clea, Tokyo, Japan), weighing 20 to 25 g, were used in these experiments. The mice were kept on laboratory chow (Central Laboratories for Experimental Animals, Tokyo, Japan) and water ad libitum.

Application of MCA. The mice were laparotomized under ether anesthesia. No. 20 cotton string coated with a mixture of MCA (Sigma Chemical Co., St. Louis, Mo.) and beeswax (Maruishi Pharmaceutical Co., Osaka, Japan), 1:3 (w/w), was inserted bilaterally into the uterine cavity by the method of Taki and Iijima (21). The mice were randomly separated into untreated and progesterone-treated groups after the implantation of MCA. The latter was further divided into 4 groups (3 low-dose groups and 1 high-dose group).

Treatment with the Low Dose of Progesterone. Progesterone (Sigma) was dissolved in sesame oil (Maruishi) at a concentration of 10 mg/ml. Each low-dose (total, 2.5 mg) group was given injections of 0.5 mg progesterone s.c. 5 times at 12-hr intervals. Each mouse in the low-dose group received the injections at the fourth week after MCA application. The other low-dose groups received the injections at the 7th and 20th week, respectively, after MCA application whether or not the tumors were palpated.

Treatment with the High Dose of Progesterone. The mice in the high-dose (total, 35 mg) group were given injections of 5 mg progesterone s.c. 7 times at 24-hr intervals. In this group the treatment was initiated as soon as tumors were palpated. These times varied from the 21st to the 40th week after MCA application.

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3 The abbreviations used are: MCA, 3-methylcholanthrene; LI, labeling index.
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Serial Labeling with [3H]Thymidine. [3H]Thymidine (specific activity, 5.0 Ci/m mole) was obtained from New England Nuclear, Boston, Mass. Each mouse in the untreated and the progesterone-treated groups was given injections of 1 μCi [3H]thymidine per g body weight 4 or 5 times at 5-hr intervals. These pulses were spaced so that [3H]thymidine labeled a maximal number of cells entering the S phase of the cell cycle, over a 15-hr or 20-hr period of time. All injections of [3H]thymidine, except the last i.p. injection given 30 min prior to sacrifice, were given i.m. to the mice.

The serial pulse labeling with [3H]thymidine described above was initiated for mice in the low-dose group immediately after each mouse had received a total dose of 2 mg progesterone (Chart 1). The control (untreated) mice were pulsed with [3H]thymidine at these times. In the high-dose group, pulsing was initiated immediately after the mice had received a total dose of 35 mg progesterone (Chart 1). The control mice were pulsed with [3H]thymidine when tumors were palpated.

Autoradiography and Thymidine-LI. The mice were sacrificed by cervical dislocation. Uterus and tumor were divided into small fragments (approximately 0.5 x 0.5 cm), fixed in Carnoy’s fluid, embedded in paraffin, and cross-sectioned at 4 to 5 μm. Autoradiographs were prepared by procedures described previously (10). At least 1000 uterine epithelial cells, stromal connective tissue cells (17), and cancer cells per mouse were counted to estimate the percentage of labeled cells. This value was defined as the LI.

Classification of Endometrial Hyperplasia and Adenocarcinoma. The “scoring” method as described by Taki et al. (22) was used to classify endometrial hyperplasia. By this method, epithelial changes are scored according to 5 criteria: (a) anisocytosis and anisokaryosis, (b) pale eosinophilic cytoplasm, (c) coarse appearance of nuclear chromatin, (d) derangement of cell layer, and (e) distortion of glandular architecture. Each of the 5 features is divided into mild and severe and assigned numerical values of 1 and 2 points, respectively.

Epithelium with only 1 of the 5 criteria (1 to 2 points) is still considered to be in the normal range. If 2 to 4 criteria (3 to 8 points) are present, the epithelium is classified as nonatypical hyperplasia. For classification as atypical hyperplasia, all 5 criteria (5 to 10 points) must be present.

In this study, atypical hyperplasia was further classified into moderate and marked. Moderate atypical hyperplasia corresponded to a total of 5 to 8 points. Marked atypical hyperplasia corresponded to a total of 9 to 10 points.

Endometrial adenocarcinoma was histologically classified into undifferentiated and differentiated. Uterine sarcoma and squamous cell carcinoma were excluded from this study.

RESULTS

Sixteen of 65 mice in the untreated group and 23 of 71 mice in the progesterone-treated (low-dose) group developed atypical hyperplasia or adenocarcinoma of the endometrium up to the 20th week of MCA application. The percentage of mice bearing atypical hyperplasia and adenocarcinoma in the progesterone-treated (low-dose) group was not significantly different (p = 0.975 by χ² test) from that of mice in the untreated group.

There was a progressive increase of endometrial hyperplasia and adenocarcinoma as a function of time (Chart 2). In contrast to the case of nonatypical hyperplasia, the incidence of moderate and marked atypical hyperplasia increased in parallel with the exposure time to MCA. Adenocarcinoma was observed at the 20th week.

LI of Epithelial Cells in Hyperplasia. The LI’s of hyperplasia in the untreated group and the progesterone-treated (low-dose) groups are shown in Charts 3 and 4, respectively.

Untreated Group. In the untreated group there was a wide distribution of LI values of epithelial cells in nonatypical hyperplasia. In contrast, LI values were generally 85 to 100% in moderate atypical hyperplasia, and all were 100% in marked atypical hyperplasia.

Four injections of [3H]thymidine were given to 3 untreated mice with moderate or marked atypical hyperplasia. In this

![Chart 1](image1.png)

![Chart 2](image2.png)
latter control group, there was no case of total labeling of cells (Chart 3).

**Progestrone-treated Group.** In the progestrone-treated (low-dose) group, LI’s of epithelial cells were mostly 4 to 6% in nonatypical hyperplasia (Chart 4). The mean LI value for nonatypical hyperplasia in this group was significantly lower than that in the untreated group, at the fourth and seventh week (p < 0.01 by Student’s t test). The mean LI value for moderate atypical hyperplasia in this group was also significantly lower (p < 0.05) than that of controls, at the seventh week. In the case of marked atypical hyperplasia, there was no significant difference (p > 0.3) in the mean LI value between the untreated and the progestrone-treated groups.

**LI of Stromal Connective Tissue Cells in Hyperplasia.** The mean LI value for stromal connective tissue cells was mostly below 25% in the untreated group at the 4th, 7th, and 20th week. The mean LI value in the progestrone-treated (low-dose) group was significantly higher (p < 0.01) than that in the untreated group and generally over 50% at the 4th, 7th, and 20th week, an effect that was opposite to that found for epithelial cells.

**LI of Endometrial Adenocarcinoma.** Six of 42 mice developed endometrial adenocarcinoma at the 20th week, and 24 of 46 mice developed endometrial adenocarcinoma 21 to 40 weeks after MCA application.

**Untreated Group.** Almost all cells were labeled with [3H]thymidine after 5 injections of [3H]thymidine (over a period of 20 hr) regardless of the date of autopsy (Figs. 1 and 2). The mean LI value for differentiated and undifferentiated adenocarcinoma was 98.0 ± 1.1 (S.E.) and 98.3 ± 1.7, respectively.

LI’s of 3 cases of differentiated adenocarcinoma and of 1 case of undifferentiated adenocarcinoma were 71, 72, 78, and 75%, respectively, after 4 injections of [3H]thymidine (over a period of 15 hr).

**Progestrone-treated Group.** The LI values of adenocarcinoma in this group were high regardless of the dosage of progesterone and the time of the treatment with progesterone (Figs. 3 and 4). Even in the high-dose group, the mean LI value for differentiated and undifferentiated adenocarcinoma was 93.9 ± 2.0 and 95.3 ± 3.0, respectively. In both differentiated and undifferentiated adenocarcinoma, there was no significant difference (0.1 < p < 0.2) in the mean LI value between the untreated and the progestrone-treated groups, regardless of the dosage of progesterone.

Marked degenerative changes were demonstrated in 5 cases (50%) of differentiated adenocarcinoma after the high-dose treatment with progesterone. However, cancer cells were still maximally labeled (Fig. 5).

Atypical hyperplasia concomitantly found with adenocarcinoma revealed remarkable atrophic changes with sparse labeling of cells. It was difficult to grade these cells further, after the high-dose treatment with progesterone, due to morphological and cytological alterations. Atrophic changes were much remarkable in atypical hyperplasia than in adenocarcinoma (Fig. 6).

**DISCUSSION**

It has been reported that the dormant (G₀) cells as defined by Lajtha (15) are present in normal uterine epithelial cells in mice (5, 10, 11) and rabbits (7). The ratio between a G₀ cell population and a dividing cell population, which can be changed by estrogen and progesterone, is important for the regulation of epithelial growth.

The results for the low-dose group clearly indicate that the biological behavior of nonatypical hyperplasia is different from that of marked atypical hyperplasia and adenocar-
cinoma. Moderate atypical hyperplasia may correspond to a transitional form from nonatypical hyperplasia to marked atypical hyperplasia.

As reported recently (12) the LI's of uterine epithelial cells change from approximately 5 to 100% in cycling mice, according to the estrous stages. The fluctuation of the LI values in nonatypical hyperplasia in controls (Chart 3) may reflect the response of epithelial cells to endogenous steroid hormones. The low-dose treatment with progesterone also caused a significant reduction in labeling with [3H]thymidine in nonatypical hyperplasia (Chart 4) to the level present in cycling mice (12). These observations suggest that the interchangeable cell populations (G₀ and dividing cells) that can be regulated by steroid hormones still remain in nonatypical hyperplasia.

In contrast, the LI's of marked atypical hyperplasia and adenocarcinoma were approximately 100% in both the untreated and the progesterone-treated groups. This result indicates that marked atypical hyperplasia and adenocarcinoma consist of dividing cells that do not respond to progesterone with mitotic arrest.

In regard to the effect of progesterone on uterine stromal connective tissue cells, Martin and Finn (17) pointed out that progesterone acts synergistically with estrogen in stimulating stromal mitosis. In this study, progesterone stimulated the proliferation of these cells even in hyperplastic epithelium, whereas it had just the opposite effect on nonatypical epithelial cells. Boquoi and Ebner (3) reported that in mice treated with 0.4 mg 19-nor-17α-hydroxyprogestrone caproate 6 days before or after the implantation of MCA, followed by weekly injections of 0.2 mg 19-norprogestin, tumor development stopped at the metaplastic-hyperplastic stage. The present study supports their contention and suggests that progesterone may act most effectively against the invasive tendency of glandular proliferation at an early hyperplastic stage not only by arresting epithelial cell division but also by stimulating stromal mitosis.

The results for the high-dose group indicate that the effect of progesterone on cancer cells is not mitotic arrest but is morphological alterations. It is not conclusive whether marked atypical hyperplasia responds to progesterone at high doses in the same way as does normal uterine epithelium.

To the author's knowledge there are no data published thus far concerning the dosage of progesterone needed to cause regression of MCA-induced endometrial carcinoma in mice. When the dose of progesterone was increased to 10 mg (total, 30 mg), there was no further reduction in labeling of cells in adenocarcinoma (unpublished observation).

Not only the dosage but also the period of administration of progesterone may be important factors in tumor regression. It is conceivable that, if the high dose of progesterone used in this study were administered for a different time period or if higher doses of the hormone were used, not only morphological degenerative changes but also mitotic arrest would have been observed in marked atypical hyperplasia and adenocarcinoma. In the case of adenocarcinoma, the histological differentiation may be a discriminating factor in this response. The previous ultrastructural observations (9, 20) pointed out that progesterone enhances autolysis in uterine epithelial cells and cancer cells. These observations seem to be important in relation to the subcellular dynamics of cancer cell degeneration. The present study indicates that marked atypical hyperplasia and adenocarcinoma, in contrast to nonatypical hyperplasia, consist of the unique dividing cell population that does not respond to progesterone with arrest of cell division.

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Fig. 1. Undifferentiated adenocarcinoma in the untreated group. Almost all cancer cells were labeled with [3H]thymidine after serial labeling over a period of 20 hr.

Fig. 2. Differentiated adenocarcinoma in the untreated group. Almost all cancer cells possessed label after serial labeling over a period of 20 hr.

Fig. 3. Undifferentiated adenocarcinoma after the low-dose treatment with progesterone (total, 2.5 mg). Labeling of cancer cells was not suppressed with the treatment. Labeling period, 20 hr.

Fig. 4. Differentiated adenocarcinoma after the low-dose treatment with progesterone. No suppression of labeling of cancer cells was demonstrated. Labeling period, 20 hr.

Fig. 5. Differentiated adenocarcinoma after the high-dose treatment with progesterone (total, 35 mg). Cancer cells still possessed label. Note remarkable morphological alterations with degenerative changes. Labeling period, 20 hr.

Fig. 6. Atypical hyperplasia after the high-dose treatment with progesterone. Marked atrophic changes with sparse labeling of cells were demonstrated. Labeling period, 20 hr.

Figs. 1 to 6. H & E, × 400.
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