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EFFECT OF DEXAMETHASONE ON UTERINE CELL DEATH

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Summary—Oestrogen, progesterone and androgen inhibit uterine cell death after the depletion of oestrogen. In the present study, we investigated effects of glucocorticoid on death of mouse uterine cells. Castrated female mice were given a daily injection of 17β-oestradiol (0.2 μg/mouse/day) for 3 days, and then an injection of 5′-[^{125}I]iodo-2′-deoxyuridine ([^{125}I]IdUrd) to label DNAs of uterine cells with ^{125}I. Mice were killed at intervals during subsequent treatments, and the retention of [^{125}I]IdUrd incorporated into the whole uterus was determined. On subsequent injection of vehicle only, the ^{125}I-radioactivity retained in the whole uterus rapidly decreased. Injections of dexamethasone (50 μg/mouse/day) reduced the loss of ^{125}I-radioactivity slightly but significantly. Dexamethasone also showed synergistic effects on the retention of ^{125}I-radioactivity when it was daily injected together with 17β-oestradiol, progesterone or 5α-dihydrotestosterone. The present results suggest that glucocorticoid may affect the processes involved in the uterine cell death, in a manner such as inhibiting the uterine cell death or delaying the removal of DNAs of dead cells from the uterus.

INTRODUCTION

The maintenance of cells after their proliferation is one of the important actions of steroids in their target organs [1–11]. In the uterus, the effects of oestrogen, progesterone and androgen on cell death have been studied and it has been shown that they inhibit cell death of epithelium [5–11].

Glucocorticoid has been shown to antagonize many oestrogen-stimulated responses in the uterus [12, 13] and to inhibit the oestrogen-independent proliferation of uterine epithelial cells [14]. However, effects of glucocorticoid on death of uterine cells has not yet been investigated. In the present study, we studied effects of glucocorticoid on death of uterine cells after the depletion of oestrogen.

EXPERIMENTAL

Chemicals

The chemicals were obtained from the following sources: 5′-[^{125}I]iodo-2′-deoxyuridine ([^{125}I]-IdUrd; 2200 Ci/mmol) and [methyl-^{3}H]thymidine (81 Ci/mmol) from Amersham International plc (Bucks, England), and fluorodeoxyuridine, 17β-oestradiol, progesterone, 5α-dihydrotestosterone, dexamethasone, Polysorbate 80 and carboxymethylcellulose from Sigma Chemical Co. (St Louis, Mo., U.S.A.).

Mice

Female C57BL/6 mice 50–60 days old were obtained from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). All the mice were castrated at the age of 60 days and used for experiments 28 days after the castration. Mice were kept at 25°C under a controlled light condition (12 h light/12 h darkness) and allowed free access to water and pellet food.

Injection of steroids

Steroids were suspended in 0.1 ml of vehicle (0.9% NaCl, 0.4% polysorbate 80, 0.5% carboxymethylcellulose, and 0.9% benzyl alcohol) and injected into the mice s.c.

Retention of [^{125}I]IdUrd incorporated into the whole uterus

Castrated female mice were daily injected with 17β-oestradiol (0.2 μg/mouse/day) at 6 p.m. for 3 days, and at 9 a.m. on the day following the last injection of 17β-oestradiol (day 1), all the

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mice were given an i.p. injection of 8 nmol/g body wt of fluorodeoxyuridine to inhibit the endogenous thymidine synthesis. After 1 h, 0.12 μCi/g body wt of [^{125}I]IdUrd was injected i.p. Six mice were killed 4 h after the [^{125}I]IdUrd injection, and the ^{125}I-radioactivity incorporated into the whole uterus was determined. The remaining mice were divided into several groups and were daily injected at 6 p.m. with vehicle only or steroid(s). Mice were killed on the morning following the last injection at intervals during the treatments, and the radioactivity retained in the whole uterus was determined as described previously [3, 10, 15]. The radioactivity retained in the whole uterus was expressed as the mean percentage of the injected radioactivity.

Effect of dexamethasone on the apoptotic index

Castrated female mice were daily injected with 17β-oestradiol (0.2 μg/mouse/day) at 6 p.m. for 3 days. On the day following the last injection of 17β-oestradiol (day 1), six mice were killed and the uterus was removed. The remaining mice were divided into 2 groups and were daily injected with vehicle only or dexamethasone (50 μg/mouse/day) at 6 p.m. from day 1. Five or six mice of each group were killed on the morning following the last injection at intervals during the treatments, and the uterus was removed. All the uteri were fixed in 10% buffered formalin and embedded in paraffin. The transverse sections (5 μm thick) of the mid portion of the uterus were prepared and stained with hematoxylin and eosin, and the apoptotic index (percentage of apoptotic cells in total cells) was determined as described previously [3, 10].

Statistical analysis

The statistical significance ($P < 0.05$) was determined by Student's t-test.

RESULTS

Effect of dexamethasone on the retention of [^{125}I]IdUrd incorporated into the whole uterus

Castrated female mice which had been daily injected with 17β-oestradiol (0.2 μg/mouse/day) for 3 days were injected with [^{125}I]IdUrd on the day following the last injection of 17β-oestradiol (day 1), and the retention of [^{125}I]IdUrd incorporated into the whole uterus was determined after the daily treatment with vehicle only or dexamethasone (50 μg/mouse/day). The uterine weight on day 1 was about 51 mg (Fig. 1). However, on injection of vehicle only, the uterine

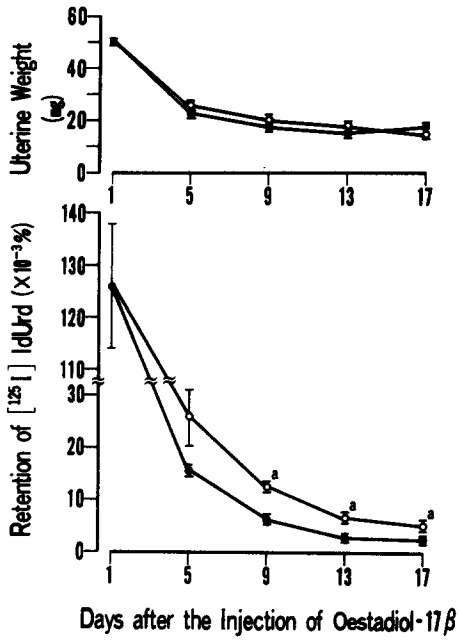

Fig. 1. Effects of dexamethasone on the uterine weight and the retention of [^{125}I]IdUrd incorporated into the whole uterus. Castrated female mice were daily injected with 17β-oestradiol (0.2 μg/mouse/day). After 3 injections of 17β-oestradiol, all mice were injected with [^{125}I]IdUrd. Mice were divided into 2 groups, and were daily injected with vehicle only (●) or dexamethasone at a dose of 50 μg/mouse/day (○). Day 1 is the day when injections of vehicle or dexamethasone were started. Each point represents the mean ± SE in 5–8 mice. $^{a}P < 0.01$ compared to the value of the mice injected with vehicle only.

weight decreased rapidly and was about 17 mg on day 17. Injections of dexamethasone had no significant effect on the uterine weight. Injections with vehicle only resulted in a marked loss of the ^{125}I-radioactivity retained in the uterus until day 13 and there was no significant loss of the ^{125}I-radioactivity thereafter (Fig. 1). The ^{125}I-radioactivity also decreased after injections of dexamethasone, but the decrease was slightly but significantly less than that on treatment with vehicle only after day 5.

[^{3}H]Thymidine (81 Ci/mmol) at a dose of 60 μCi/mouse was injected into castrated female mice instead of [^{125}I]IdUrd and then mice were daily injected with dexamethasone (50 μg/mouse) or vehicle only. Mice were killed after 12 daily injections and the autoradiography of uterine transverse sections was carried out. In the uterine lumens of mice treated with dexamethasone or vehicle only, the distribution of grains was the same as that in the background around the uterus and no apoptotic cells were found. However, concentrated grains were found in the uterine tissue including epithelium and stroma (data not shown).

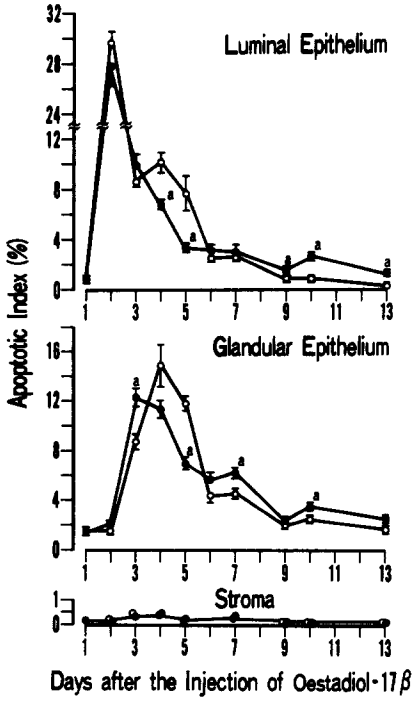

Fig. 2. Effect of dexamethasone on the apoptotic index of mouse uterine cells. Experimental protocol was the same as that for Fig. 1 except that [^{125}I]IdUrd was not injected into mice. Mice of one group (●) were injected with vehicle only and mice of the other (○) with dexamethasone (50 μg/mouse/day). Each point represents the mean ± SE in 5–6 mice. $^{a}P < 0.05$ compared to the value of mice injected with dexamethasone.

The effect of dexamethasone on the apoptotic index (percentage of apoptotic cells in total cells) was investigated in a similar experimental protocol but without the injection of [^{125}I]IdUrd (Fig. 2). The apoptotic indices of luminal and glandular epithelia were low on day 1. On treatment with vehicle only, apoptotic indices of luminal and glandular epithelia increased markedly on days 2 and 3, respectively, and decreased gradually thereafter. On treatment of dexamethasone, the apoptotic index of luminal epithelium was higher on days 4 and 5 but lower after day 7 compared to that on treatment with vehicle only. Injections of dexamethasone delayed the increase in the apoptotic index of glandular epithelium and slightly lowered its apoptotic index after day 6. The apoptotic index of stroma was extremely low during the treatment with vehicle, and injections of dexamethasone had no significant effect on it.

Effects of dexamethasone on the retention of [^{125}I]IdUrd were examined when dexamethasone was injected together with 17β-oestradiol, progesterone or 5α-dihydrotestosterone. Injections of 17β-oestradiol (0.2 μg/mouse/day), progesterone (1 mg/mouse/day) and 5α-dihydrotestosterone (100 μg/mouse/day) for 15 days increased the retention of [^{125}I]IdUrd about 2.6-, 6.6- and 6.5-fold, respectively (Table 1). Injections of dexamethasone at doses more than 5 μg/mouse/day caused synergistic effects on the retention of [^{125}I]IdUrd.

Dexamethasone also decreased the uterine wet weight increased by 17β-oestradiol or 5α-dihydrotestosterone, but had no significant effect on the uterine wet weight when injected together with progesterone (Table 2).

DISCUSSION

[^{125}I]IdUrd retained in the uterus is incorporated into DNAs of uterine cells. It has been confirmed in our previous study in which we found by measurement of the radioactivities incorporated into RNA, DNA and protein of

Table 1. Effects of dexamethasone on the retention of [^{125}I]IdUrd incorporated into the whole uterus

Dose of dexamethasone (μg/mouse/day)	Retention of [^{125}I]IdUrd ($\times 10^{-4}$ %)			
	Vehicle	E_2	Prog	DHT
0	38.6 ± 1.6	98.7 ± 5.7	257.0 ± 16.7	250.2 ± 11.9
5	ND	122.5 ± 13.5[a]	314.7 ± 16.3[a]	309.1 ± 23.5[a]
10	ND	136.9 ± 14.3[a]	317.1 ± 19.1[a]	337.8 ± 21.5[a]
20	ND	152.4 ± 12.3[a]	320.6 ± 19.5[a]	323.4 ± 26.3[a]
50	64.1 ± 6.7[a]	204.5 ± 17.9[a,b,c,d]	385.9 ± 27.8[a,b]	370.0 ± 13.9[a,b,c,d]

Castrated female mice were daily injected with 17β-oestradiol (0.2 μg/mouse/day) for 3 days. On the following day after the last injection of 17β-oestradiol, all mice were injected with [^{125}I]IdUrd and divided into several groups. Then, mice were injected daily with dexamethasone at various doses or with dexamethasone at various doses plus 17β-oestradiol (E_2, 0.2 μg/mouse/day), progesterone (Prog, 1 mg/mouse/day) or 5α-dihydrotestosterone (DHT, 100 μg/mouse/day) for 15 days. All mice were killed on the day following the last injection. ND: Not determined.

[a] $P < 0.05$ compared to the value of mice injected without dexamethasone.

[b] $P < 0.05$ compared to the value of mice injected together with dexamethasone at a dose of 5 μg/mouse/day.

[c] $P < 0.05$ compared to the value of mice injected together with dexamethasone at a dose of 10 μg/mouse/day.

[d] $P < 0.05$ compared to the value of mice injected together with dexamethasone at a dose of 20 μg/mouse/day.

Table 2. Effects of dexamethasone on the uterine weight

Dose of dexamethasone (μg/mouse/day)	Uterine wet wt (mg)			
	Vehicle	E_2	Prog	DHT
0	16.5 ± 0.6	79.9 ± 2.4	31.5 ± 0.9	73.3 ± 1.1
5	ND	63.9 ± 3.1*	30.0 ± 1.0	66.8 ± 1.1*
10	ND	69.6 ± 2.9*	30.3 ± 1.1	62.1 ± 3.1*
20	ND	64.6 ± 1.9*	30.6 ± 1.1	65.6 ± 2.6*
50	15.8 ± 0.6	61.6 ± 1.7*	33.7 ± 1.9	61.4 ± 1.8*

The experimental protocol was shown in Table 1. E_2: 17β-oestradiol (0.2 μg/mouse/day); Prog: progesterone (1 mg/mouse/day); DHT: 5α-dihydrotestosterone (100 μg/mouse/day). ND: not determined.

*$P < 0.05$ compared to the value of mice injected without dexamethasone.

the uterus that more than 90% of the ^{125}I-radioactivity was incorporated into the DNA fraction [15]. Thus, the loss of the ^{125}I-radioactivity in the whole uterus reflects the loss of ^{125}I-labelled DNAs from the uterus.

Dexamethasone decreased the loss of the ^{125}I-radioactivity retained in the whole uterus. As shown by the autoradiographic study, the radioactivity retained in the uterus remains not in the uterine lumen but in the uterine tissue. Thus, this result suggests that dexamethasone might affect the processes involved in the uterine cell death in a manner such as inhibiting the uterine cell death or delaying the removal of DNAs of dead cells from the uterine tissue. Dead cells are extruded into an adjacent lumen, phagocytosed by residual tissue cells and ingested by mononuclear phagocytes [2]. Since the functions of mononuclear phagocytes are modulated by glucocorticoid [16, 17], the effect of dexamethasone on the removal of DNAs of dead cells from the uterus is possible.

Dexamethasone showed synergistic effects on the retention of [^{125}I]IdUrd incorporated into the whole uterus when injected together with oestrogen, progesterone and 5α-dihydrotestosterone. These steroids principally inhibit the cell death of uterine epithelium [5, 7–11]. Thus, it is likely that dexamethasone may affect the processes involved in the cell death of uterine epithelium. In the study about effects of dexamethasone on the apoptotic index, which is a good quantitative index of physiological cell death [3, 5, 7–11], some effects of dexamethasone on the apoptotic index of epithelium were observed, although the effects varied depending on days of the treatment. This result may further support the above-mentioned presumption.

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