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Abstract

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KEYWORDS: steroid sex hormones, human urinary bladder cancer cell line, cytotoxic effect, adriamycin

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EFFECTS OF STEROID SEX HORMONES AND ADRIAMYCIN ON HUMAN BLADDER CANCER CELLS IN CULTURE

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Abstract. The effects of steroid sex hormones on the established cell lines derived from human urinary bladder cancer, T24, and from human transitional cell cancer of the urinary tract, 253J, were examined using the colony formation method. Of the seven kinds of steroid hormones tested, estradiol-17β was intensively cytotoxic for both cells. The cytotoxic effect was depended on the dose and time of treatment. The combined effect of Adriamycin and estradiol-17β on T24 cells could be recognized at low concentrations of Adriamycin ($\leqq 10^{-3}$ μg/ml) after exposure for 24 h.

Key words : steroid sex hormones, human urinary bladder cancer cell line, cytotoxic effect, adriamycin.

In patients with superficial bladder tumors, various anticancer drugs have been instillated in the bladder since Jones and Swinney reported that Thio-Tepa was effective against bladder tumors when given intravesically (1). Because of problems such as bladder irritation and a high recurrence rate, however, development of more effective drugs with fewer side effects is needed (2, 3). Furthermore, it is known that males have a higher incidence of bladder tumors than females. Kono *et al.* suggested that testosterone was a possible promotor in experimental bladder tumors induced in Wistar-Imamichi strain male rats by oral administration of 0.05% N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) (4). However, there have, as yet, been no reports of treatment with steroid sex hormones, nor have the effects of steroid sex hormones on human bladder cancer cells *in vitro* been examined.

In this study, the effects of various sex steroid hormones on the established cell line T24 derived from human urinary bladder cancer (5) and 253J from human transitional cell cancer of the urinary tract (6) were examined using the colony formation method. The effect of a combination of Adriamycin (ADM) and estradiol-17β (E_2) was also studied using T24 cells with the aim of improving the therapeutic results for treatment of bladder tumors by the use of steroid sex hormones.

MATERIALS AND METHODS

Cell cultures. Established cell lines, T24, derived from human urinary bladder cancer, and 253J, from human transitional cell cancer of the urinary tract, were used. The donor of the T24 cells was an 82-year-old female patient with long-diagnosed urinary bladder papillomatosis. The donor of the 253J cells was a 53-year-old male who had a 2-year history of multiple massive transitional cell neoplasms of the bilateral renal pelvis, ureters, and urinary bladder. A rat bladder tumor, RBC cells induced by BBN *in vivo* (7), and a rat hepatoma, dRLa-74 cells induced by 4-(dimethylamino) azobenzene (DAB) *in vivo* (8) were used as the control.

Cells were maintained as monolayer cultures in Eagle's MEM (Nissui Pharm. Co. Ltd., Tokyo, Japan) supplemented with 20 % heat-inactivated bovine serum and 200 u./ml penicillin. The cells were grown at 37℃ supplied with humidified 5 % carbon dioxide and 95 % air. At subculturing, T24 cells, RBC cells and dRLa-74 cells were dispersed with 0.2 % trypsin, and 253J cells were dispersed with 0.1 % trypsin + 0.05 % EDTA.

Hormones. Six kinds of steroid sex hormones: progesterone (P), estrone (E_1), estradiol-17β (E_2), estriol (E_3), testosterone (T), and dehydroepiandrosterone sulfate (DHEA-S), with cortisol (F) as the control were given to T24 cells and 253J cells.

All of the steroids were purchased from the Sigma Chemical Co., St. Louis, U.S.A.. Steroid sex hormones were dissolved in dimethyl-sulfoxide (DMSO) and stored at 4℃ until use. F was dissolved in DMSO immediately before use. The hormones were diluted with culture medium and added to give final concentrations ranging from 5 to 160 μg/ml. The control cultures were kept with the same amount of 1 % DMSO having no hormones.

Chemotherapeutic agent. ADM (Kyowa Hakko, Tokyo, Japan) was dissolved in culture medium immediately before use. Treatment of cells with ADM was performed by replacing the growth medium with medium containing graded concentrations (0.0001 to 10 μg/ml) of ADM.

Treatment of cells with hormones. In this experiment, the cytotoxic test was done by treatment with hormones of plated single cells capable of giving rise to colonies. Monolayers of T24 and 253J cells were harvested with trypsin or trypsin/EDTA. For each experiment, 100 viable cells suspended in 3 ml culture medium were plated in 60 mm plastic dishes (Falcon, Calif.). After 24 h of incubation, the culture medium was replaced by medium with or without hormones, and kept for 24 h or 48 h at 37℃. After the treatment was terminated, the cultures were rinsed twice with Hank's balanced salt solution, then replaced with fresh medium. After 6 to 7 days of incubation in the case of T24 cells, and 8 to 9 days in the case of 253J cells in humidified 5 % carbon dioxide and 95 % air, the cultures were fixed in methanol and stained with Giemsa. The experiment was conducted twice in triplicate.

Combined treatment of cells with ADM and E_2. T24 cells were used because these cells had a higher plating efficiency than 253J cells. T24 cells were incubated for 2 h or 24 h in medium containing graded concentrations of ADM (0.00005 to 5 μg/ml) and E_2 (5 to 80 μg/ml), or containing graded concentrations of ADM (0.0001 to 10 μg/ml) or E_2 (10 to 160 μg/ml) alone. Control cultures contained the same amount of 1 % DMSO. The experiment was conducted three times in triplicate.

RESULTS AND DISCUSSION

As shown in Fig. 1, the number of colonies formed by T24 cells decreased

significantly with the use of E_2 ($p < 0.001$), decreased moderately with P and E_3, and decreased slightly with E_1, T, DHEA-S and F when exposed for 24 h. Similar results were obtained for 253J cells, although not shown in data.

The cytotoxic effect of E_2 on these cells was dependent on time as shown in Fig. 2 as well as on dose. The 50 % inhibitory time of 80 μg/ml of E_2 on colony formation was 8 h for 253J cells and 14.4 h for T24 cells. It is difficult to explain the mechanism of such a specific cytotoxic effect of E_2 on T24 cells and 253J cells. Hormone receptors, although specific sites associated with urinary bladder cancer cells have never been confirmed, may be involved in the mechanism as suggested by the experiments of Sekiya *et al.* using rat uterine adenocarcinoma cells (9).

Furthermore, the effects of 80 μg/ml of E_2 on the plating efficiency of T24, 253J, RBC and dRLa-74 cells were compared. E_2 was more cytotoxic for both T24 and 253J cells than for RBC cells, and for RBC cells more than for dRLa-74 cells (Table 1). These results suggest that the cytotoxic effects of E_2 on T24

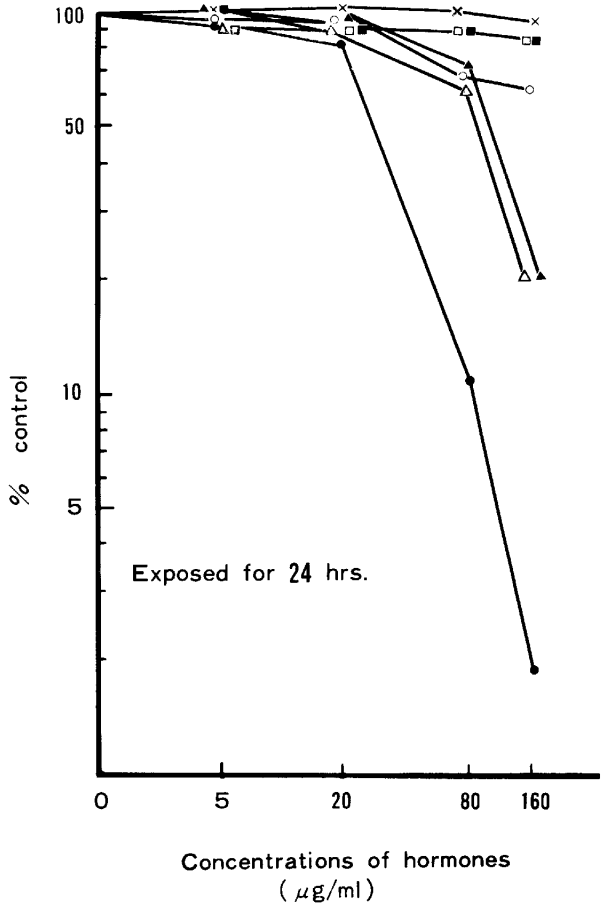

Fig. 1. Effects of steroid hormones on the plating efficiency of T24 cells. ●——● Estradiol-17β (E_2), ○——○ Estrone (E_1), ▲——▲ Estriol (E_3), △——△ Progesterone (P), ■——■ Dehydroepiandrosterone sulfate (DHEA-S), □——□ Testosterone (T), ×——× Cortisol (F). The 50 % inhibitory dose of E_2 on colony formation of T24 cells was 35 μg/ml and that of 253J cells was 16.25 μg/ml.

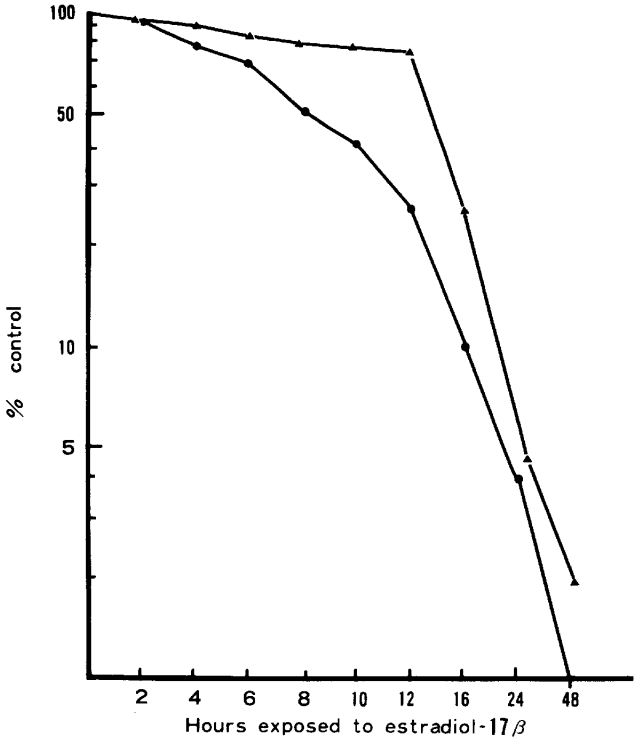

Fig. 2. Effect of 80 μg/ml of estradiol-17β at various times of exposure on colony formation of T24 cells & 253J cells. ▲——▲ T24 cell line, ●——● 253J cell line. The 50 % inhibitory time of 80 μg/ml of E_2 on colony formation was 8 h in 253J cells and 14.4 h in T24 cells.

TABLE 1. EFFECTS OF ESTRADIOL-17β (80 μG/ML) ON THE PLATING EFFICIENCY OF VARIOUS CELL LINES, 253J, T24, RBC & dRLa-74.

	Exposed time(h)	Relative plating efficiency			
		253J	T 24	RBC[a]	dRLa-74[b]
Control		100	100	100	100
Estradiol-17β	24	6.0	11.0	26.5	67.3
	48	1.0	2.0	10.0	33.6

a Derived from rat bladder tumors. *b* Derived from rat hepatomas.

and 253J cells show tissus and species specific patterns.

There was no significant difference in the cytotoxic effects of ADM alone and in combination with E_2 when exposed for 2 h, although the data are not shown. When exposed to drugs for 24 h, at low concentrations ($\leqq 10^{-3}$ μg/ml), ADM in combination with E_2 showed significantly more cytotoxic effects than ADM alone ($p<0.001 - p<0.05$). In Table 2, the combined effects were examined at a concentration of 10 μg/ml of E_2. The combined effects of ADM and E_2 could not be obtained when ADM was in high concentrations ($\geqq 10^{-2}$ μg/ml), since the cytotoxic effect of ADM was striking at such concentrations. Similar combined effects were obtained at 40 μg/ml and 160 μg/ml of E_2, although the

TABLE 2. COMBINED EFFECTS OF ADRIAMYCIN (ADM) AND ESTRADIOL-17β (E_2) ON THE PLATING EFFICIENCY OF T24 CELLS

	Relative plating efficiency
Control	100
E_2 10 μg/ml	85.3 ± 2.5[a]
ADM 10^{-4} μg/ml	94.5 ± 6.4
$\frac{ADM + E_2}{2}$ $\frac{10^{-4} + 10}{2}$ μg/ml	83.4 ± 0.9
ADM 10^{-3} μg/ml	83.4 ± 3.7
$\frac{ADM + E_2}{2}$ $\frac{10^{-3} + 10}{2}$ μg/ml	79.7 ± 2.5
ADM 10^{-2} μg/ml	8.8 ± 6.2
$\frac{ADM + E_2}{2}$ $\frac{10^{-2} + 10}{2}$ μg/ml	23.5 ± 5

[a] Mean ± S.E.

data are not shown.

Intravesical instillation of ADM against bladder tumors has been developed and performed on many cancer patients for the first time by us (2). Intravesical instillation of ADM in a high concentration was required to obtain good effects, and this in turn increased the incidence of side effects such as bladder irritation which was often noticed as in the case of other anticancer agents used for intravesical instillation (2, 10, 11). Therefore, development of new methods involving combination therapy with ADM needs to be considered.

Holmes *et al.* have stated that drug effects on cultured cells parallel the clinical effects (12) although their experiments were conducted using primary cultured cells, but no cell lines. The results obtained in the present experiments suggest that intravesical instillation of E_2 alone or in combination with ADM in relatively low concentrations is useful against bladder tumors without increasing the side effects.

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