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AROMATIZATION OF ANDROSTENEDIONE BY NORMAL AND NEOPLASTIC ENDOMETRIUM OF THE UTERUS

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Summary—The ability of human uterine endometrium to aromatize androstenedione to estrogens was investigated using 10 normal and neoplastic tissues. Normal and neoplastic endometrial homogenates were incubated with [6,7-^{3}H]androstenedione (A) and NADPH. Estrone (E_1) and estradiol (E_2) were subsequently isolated in amounts ranging from 0–17600 fmol/h/g and 0–377 fmol/h/g, respectively, from the incubates after purifications by using Bio-Rad AG1-X2 column, thin layer chromatographies and co-crystallization. The conversion of A to E_1 and E_2 was significantly higher in neoplastic tissues.

INTRODUCTION

It has been established that the conversion of circulating androstenedione to estrogens in the peripheral tissue is an important source of estrogen in postmenopausal women [1, 2, 3]. The authors have reported that human abdominal adipose tissue has an ability to convert androstenedione to estrone and estradiol and this ability is enhanced significantly with increase of the total measure of adipose tissue weight and with aging [4]. Human endometrial carcinoma is closely associated with estrogen and the incidence of its appearance is high in obese women after menopause [5]. It is, however, still under debate whether or not enhanced estrogen formation in adipose tissue and other peripheral tissues is causative of the neoplastic development of endometrium.

According to the work of Tseng *et al.* [6] human endometrium itself has an ability to aromatize androgen to estrogen, indicating that it contributes to peripheral formation of estrogen. Moreover, they reported that the capacities of aromatization in both proliferative and cancer endometria were found to be significantly higher than that in secretory endometria.

However, little is yet known about the aromatizing enzyme system in human uterine endometrium. In this study, the authors investigated the conversion of androstenedione to estrone and estradiol in normal and neoplastic endometrial tissues and observed the aromatizing enzyme activity to be elevated by neoplastic transformation.

EXPERIMENTAL

Steroids

[6,7-^{3}H]Androstenedione (A; 26 Ci/mmol) and [4-^{14}C]estrone (E_1; 55.8 mCi/mmol) were purchased from the Amersham Corp. (RCC, U.K.). [4-^{14}C]Estradiol-17β (E_2; 53.9 mCi/mmol) was purchased from the New England Nuclear Corp. (Boston, MA, U.S.A.). Non-radioactive E_1 and E_2 were purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.).

Tissue preparation

Endometrial tissue samples were obtained from 20 patients at hysterectomy (10 cases of leiomyoma of the uterus, and 10 cases of endometrial cancer of the uterus). Portions of these tissues were submitted to histological examination and dating of the menstrual cycle, and the remainder were stored at −40°C until analysis. Endometrial tissues obtained from the patients with leiomyoma were used as normal endometrial samples in this study. Endometrial cancer tissues were subgrouped into the following three histological grades; a poorly differentiated type (G_3, 3 cases), a moderately differentiated type (G_2, 2 cases), a well differentiated type (G_1, 4 cases), with one case of adenosquamous cell carcinoma.

Incubation and assay of aromatase activity; A portion of endometrial tissue (500 mg w.w.) was minced and homogenized in 5 ml of 0.1 M-phosphate buffer (pH 7.4) using a glass homogenizer. The mixture of the tissue homogenate, [6,7-^{3}H]A (10 μCi, 385 pmol) and NADPH (2.5 mg) was incubated at 37°C for 4 h in air. After incubation, the enzyme reaction was terminated with the addition of 3 vol of ethyl acetate and subsequently [4-^{14}C]-E_1 and [4-^{14}C]-E_2 (10,000 dpm, 250 μg) were added as tracers in the incubate. The steroids were separated from the incubated sample by partition between ethyl acetate and water 3 times. The extracts by ethyl acetate were displaced with 1 ml of methanol and subjected to Bio-Rad AG1-X2 (50 ~ 100 mesh) chromatography [7]. The phenolic steroids thus obtained were further purified by thin layer chromatography (TLC; cyclohexane–ethylacetate = 2:1, v/v, 2 developments,

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Table 1. $^3H/^{14}C$ Ratio after repeated crystallization of isolated E_1 and E_2 in neoplastic endometrial tissues.

Case no.	Estrogen	Last thin layer	1-ML	1-Cr	2-ML	2-Cr	3-ML	3-Cr
1	E_1	244	1207	683	80.9	90.5	64.0	72.6
	E_2	10.4	8.89	0.57	1.71	0.27	0.32	0.23
4	E_1	0.42	0.82	0.24	0.28	0.23	0.28	0.26
	E_2	3.79	5.58	0.12	0.58	0.07	0.07	0.06
6	E_1	0.58	1.59	0.24	0.55	0.28	0.13	0.16
	E_2	3.39	4.51	0.14	0.37	0.00	0.00	0.00
7	E_1	1.39	2.40	2.45	2.00	1.87	1.50	1.45
	E_2	0.35	0.42	0.45	0.35	0.27	0.25	0.24
8	E_1	3.24	3.13	3.03	2.59	3.02	3.28	3.54
	E_2	4.33	1.06	0.78	0.94	0.68	0.77	0.73

ML: mother liquor.
Cr: crystal.

and chloroform–ethyl ether = 4:1, v/v, 1 development). The resulting E_1 and E_2 were subjected to co-crystallization to constant specific activity and $^3H/^{14}C$ ratio of the crystals, respectively. The conversion rate from androstenedione to estrogen (aromatase activity) was expressed as fmol of estrogen formed per h per g of tissue. These analytical procedures are reported elsewhere [8, 9].

RESULTS

The purity of isolated estrogen was identified by co-crystallization with authentic steroid to check the consistency of $^3H/^{14}C$ ratio (Table 1).

The conversion of A to E_1 and E_2 (aromatase activity) in the normal endometrium of the uterus is presented in Table 2. The amounts of E_1 produced by endometrial tissues in the proliferative and ovulatory phases were in the range of 27–131 fmol/h/g while corresponding values for E_2 were in the range of 0–228 fmol/h/g. On the contrary, the amounts of estrogen produced by endometrial tissues in the secretory phase were in the range of 0–50 fmol/h/g and 0–15 fmol/h/g for E_1 and E_2, respectively. Higher levels of aromatase activity for androstenedione were observed by the endometrial tissues in the proliferative and ovulatory phases [$P = 0.05$, (E_1, E_2)] compared to those tissues in the secretory phase.

Table 3 presents the amounts of estrogen produced per h per g tissue in the endometrial cancer tissues. In tissues of cases 1–3 with poorly differentiated adenocarcinoma, 156–17600 fmol of E_1 and

Table 2. Estrogen formation in normal endometrium.

Case no.	Age (yr)	Menstrual cycl.	Estrogen formation (fmol/h/g) Estrone	Estradiol
1	46	P.P.	27	N.D.
2	40	P.P.	70	72
3	36	O.P.	131	228
4	42	S.P.	34	N.D.
5	45	S.P.	13	N.D.
6	49	S.P.	50	15
7	53	S.P.	22	15
8	46	S.P.	39	13
9	49	S.P.	37	N.D.
10	37	S.P.	N.D.	N.D.

Normal endometrial tissue homogenates (500 mg w.w.) prepared in 5 ml of 0.1 M-phosphate buffer (pH 7.4) were mixed with 10 μCi of [6,7-^{3}H]-androstenedione (385 pmol) and NADPH (2.5 mg). The mixtures were incubated at 37°C for 4 h in air. Estrogen produced in human uterine endometrium was assayed after purification involving Bio-Rad AG1-X2 column chromatography, TLC and co-crystallization.
N.D.: not detected (under 10 fmol/h/g).
P.P.: proliferative phase.
O.P.: ovulatory phase.
S.P.: secretory phase.

Table 3. Estrogen formation in neoplastic endometrium. The procedures were identical to those described in Table 2.

Case no.	Age (yr)	Histological grade	Estrogen formation (fmol/h/g) Estrogen	Estradiol
1	50	G_3	17600	59
2	59	G_3	156	231
3	62	G_3	327	47
4	47	G_2	66	17
5	66	G_2	323	377
6	70	G_1	36	N.D.
7	58	G_1	169	61
8	67	G_1	42	30
9	53	G_1	620	165
10	68	adenosq. cell ca.	412	100

N.D.: not detected (under 10 fmol/h/g)

47–231 fmol of E_2 were converted from A, which were much higher than the values obtained with normal endometrial tissues. Tissues of cases 4 and 5 with moderately differentiated carcinoma produced 66–233 fmol and 17–377 fmol of E_1 and E_2, respectively. 36–620 fmol of E_1 and 0–165 fmol of E_2 were isolated in cases 6–10 with well differentiated carcinoma. The mean values of E_1 and E_2 produced in these neoplastic groups were 1975 fmol and 121 fmol, respectively. Consequently, moderately (G_2) and well differentiated carcinoma tissues (G_1) exhibited a relatively high level of aromatase activity compared to normal tissues. Moreover, aromatase activity for androstenedione in poorly differentiated carcinoma (G_3) was remarkably high even when compared to other neoplastic tissues (G_1 and G_2).

These results demonstrate that human endometrial tissues possess the capacity for aromatizing androstenedione to estrogens, irrespective of neoplasticity. The aromatase activity in the human endometrial tissue was shown to fluctuate from low to high levels, according to the phase of the menstrual cycle or the neoplastic differential state. The conversion rate of androstenedione to estrogens was higher in the neoplastic endometrium compared to normal tissue.

DISCUSSION

Estrogen is believed to be one of the promotive factors of endometrial cancer [10, 11]. There are some reports that human endometrial cancer is frequently found in obese women after menopause [5, 12, 13]. The authors have previously investigated the aromatase activity in the adipose tissue of patients with various benign gynecological diseases and have reported that this enzyme activity increased parallel to the total measure of adipose tissue weight and to the post menopausal period [4]. The total mass of adipose tissue would be greater in obese women and consequently they are expected to possess elevated estrogen levels by the aromatization of peripheral androgen in adipose tissue.

Uterine endometrium, as well as adipose tissue, is known to contribute to the peripheral estrogen formation by the recent report. Baxendale *et al.* [14] have investigated the aromatase activity in normal human endometrium and have reported the inability of this tissue to convert androstenedione to estrone. On the contrary, Tseng *et al.* [6] have examined the aromatization of testosterone in human endometrium and detected the estrogenic products in both normal and neoplastic endometrium. In the present study, the authors also investigated estrogen biosynthesis by normal and neoplastic endometrial tissues *in vitro* and obtained the results supporting their observations that uterine endometrium is capable of aromatizing androstenedione to estrogens. This aromatizing enzyme activity was significantly higher in the neoplastic endometrium compared to normal tissue. This enhanced activity of aromatization would increase estrogen concentration in the neoplastic endometrium and thus, it may promote the cancer cell growth. Among the 3 histological subgroups of endometrial cancer studied, the enhancement of aromatase activity was most pronounced in the poorly differentiated type (G_3) rather than in the moderately or well differentiated types (G_2 and G_1). The relevant explanation to the enhanced aromatization is the matter in question. It has been agreed upon that estrogen receptors are reflected in the degree of tumor differentiation. Some investigators [15, 16, 17] have indicated the existence of a positive correlation between the decrease in cytosolic estrogen receptor and the immaturity of cell differentiation in endometrial cancer cells. They have expressed the view that the biological effects of estrogen are poorly delivered via receptor proteins to the target cells in the undifferentiated carcinoma due to this impaired mechanism of estrogenic action. So the aromatizing enzyme of androgen in these cancer cells may be activated as the result of insufficient estrogen due to the decrease in receptor proteins.

The factors that regulate estrogen synthesis in the peripheral tissue are still under debate. Folkerd *et al.* [18] examined the influence of serum LH and FSH levels on aromatase activity in adipose tissue and found no relationship between the former and the latter. The effect of glucocorticoid upon aromatase activity in human adipose tissue has also been examined by various researchers [19, 20, 21] and dexamethasone has been reported as being the most potent steroid in stimulating the enzyme activity.

The present data does not provide any information concerning the regulation of the aromatizing system in endometrial cancer. However, after menopause the aromatizing enzyme system may be stimulated by any hormonal substances such as above. It is speculated that this enhancement of estrogen production may contribute to the further growth of the neoplasia.

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