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Treatment with high-dose estrogen (diethylstilbestrol) significantly decreases plasma estrogen and androgen levels but does not influence in vivo aromatization in postmenopausal breast cancer patients☆

Jürgen Geisler[a], Ben Haynes[b], Gun Anker[a], Hildegunn Helle[a], Dagfinn Ekse[a], Mitch Dowsett[b], Per Eystein Lønning[a,*]

[a] *Department of Medicine, Section of Oncology, Haukeland University Hospital, N-5021 Bergen, Norway*
[b] *Academic Department of Biochemistry, Royal Marsden Hospital, London, UK*

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Abstract

While growth factors and hormones are known to influence aromatase expression in experimental systems, little is know about potential factors influencing peripheral aromatization in postmenopausal women. The fact that peripheral aromatase activity is higher in old compared to young women and the finding of relatively high tissue estradiol (E_2) concentrations after the menopause suggests peripheral aromatization could be influenced by estrogen concentration. To test this hypothesis, we determined plasma hormone levels ($n = 9$) and in vivo aromatization ($n = 3$) in postmenopausal women suffering from advanced breast cancer before and during treatment (4 weeks) with diethylstilbestrol (DES) 5 mg three times daily. Plasma levels of cortisol (C), corticosteroid-binding globulin (CBG), and sex hormone binding globulin (SHBG) were significantly increased in all patients ($P < 0.05$ for all). While we found no change in total body aromatization and plasma estrone (E_1) levels, estradiol (E_2) and estrone sulfate (E_1S) were suppressed by a mean of 48.8 and 68.2%, respectively ($P = 0.043$ and 0.008). Surprisingly, plasma levels of androstenedione (A), testosterone (T), dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) were also suppressed by a mean in the range of 32.1 to 52.6% ($P < 0.05$ for all androgens). In contrast, no change in plasma progesterone or 17α-hydroxyprogesterone was found. Thus, one possible explanation to our findings could be that DES administered in high doses reduces 17,20-lyase activity in the adrenal gland.

Keywords: Breast cancer; Aromatase; Diethylstilbestrol; Estrogens; Androgens; 17,20-Lyase

1. Introduction

Non-glandular estrogen synthesis [1–3] plays a key role in many physiological processes in both sexes [4–6] and represents a major target for endocrine therapy in postmenopausal breast cancer [7,8]. The rate-determining enzyme for estrogen synthesis in postmenopausal women is aromatase. While only one aromatase gene has been identified, transcription is regulated in a tissue-specific manner by the use of alternative promoters [9,10]. Although multiple growth factors, interleukins and glucocorticoids have been found to regulate aromatase expression in experimental systems [11–17], little is known about potential regulatory mechanisms of non-glandular aromatization in vivo.

Estrogen disposition in postmenopausal women is incompletely understood. While glandular hormone production ceases at menopause, causing a substantial drop in circulating estrogen levels, tissue estrogens (in particular E_2 levels) are better preserved in both benign and malignant tissues [18]. Fibroblasts isolated from old women are known to express higher levels of aromatase compared to cells obtained from young individuals [19], and one study found total body

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* Corresponding author. Tel.: +47 55 97 2010; fax: +47 55 97 2046.
E-mail address: per.lonning@helse-bergen.no (P.E. Lønning).

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aromatization to be higher in postmenopausal compared to premenopausal women [20]. While these findings are consistent with direct or indirect product feedback control of peripheral aromatase activity, this hypothesis has never been proved.

In a recent study, we evaluated the therapeutic benefits of high-dose estrogens in postmenopausal breast cancer patients exposed to previous endocrine manipulation [21]. To test the hypothesis that exogenous estrogens could influence in vivo aromatization, we measured plasma hormone levels and whole body aromatization in vivo in subgroups of women ($n = 9$ and $n = 3$, respectively) before and during (4 weeks) treatment with diethylstilbestrol (DES) 5 mg three times daily.

2. Methods

2.1. Patients and treatment

Nine postmenopausal women with metastatic breast cancer eligible for treatment with DES due to progressive disease were enrolled in this protocol (Table 1). The median age was 72 years (range 53–87 years). Six of the patients were included in our series reported for clinical efficacy [21]. The patients were treated with diethylstilbestrol (DES) given orally at a dose of 5 mg three times daily. Any other treatment with compounds that might influence plasma steroids was terminated at least 4 weeks prior to the baseline blood samples. The protocol was approved by the Regional Ethical Committee.

Due to the results, we wished to evaluate whether postmenopausal estrogen replacement therapy could also influence plasma androgen levels. Thus, plasma samples obtained prior to and during treatment from 14 patients participating in a previous study comparing the effects of estradiol 2 mg p.o. versus 50 μg/24 h transdermally [22] were analyzed for androstenedione and testosterone levels as one part of this study.

2.2. Plasma samples

Plasma samples were drawn prior to initiation of treatment and following 4 weeks on treatment with DES. All plasma samples were obtained following an overnight fast between 08:00 and 09:00 a.m. in heparinized vials. The samples were centrifuged and stored at −20 °C until processing.

2.3. Plasma estrogen levels

Plasma estrone (E_1) and estradiol (E_2) levels were determined by highly sensitive radioimmunoassays (RIA) established at our laboratory as published previously [23,24]. Plasma estrone sulfate (E_1S) levels were determined by a novel, highly sensitive assay involving purification and derivatization into E_2 and by RIA analysis using estradiol-6-carboxymethyloxime-[2-^{125}I]-iodohistamine as tracer ligand [25]. The sensitivity limits for plasma levels of E_2, E_1 and E_1S were 2.1, 6.3, and 2.7 pmol/l, respectively. Cross-reactivity for DES in our E_2 and our E_1 method was 0.003 and 0.007%, respectively. Taking into account the concentration of DES in the plasma expected during treatment with DES 5 mg three times daily (approximately 30 ng/ml) [26], these cross-reactions are negligible and not measurable in our assays. To test for potential interactions from DES metabolites in plasma, we re-analyzed samples from four patients for E_2 and E_1S and from all patients for E_1 following HPLC-purification as described for tissue estrogen analysis elsewhere [27]. No differences in the steroid concentrations measured either with or without HPLC-purification were recorded. To test for possible influences of high SHBG levels in our estrogen assays, we added known concentrations of unlabelled estrogens to our plasma samples obtained prior to and during DES therapy. Our tests revealed no influence of increasing SHBG levels on plasma estrogen levels. The recovery of added estrogens was exactly as calculated upfront for samples with normal SHBG levels and those with SHBG levels increased by 4–500% by DES.

Table 1
Patient characteristics

Patient	Age	ER	PGR	CT[a]	ET	Responses (ET)
1	87	+	+	5/5	T, M, F, F + AG, T	PR, PR, SD, PR, SD
2	65	+	+	2/2	T, M, AG, T	SD, CR, PR, PD
3	74	+	–	1/2	T, E, M, T, A	PR, SD, PD, SD, SD
4	68	+	+	0/1	M, AG	SD, SD
5	72	+	+	2/3	T, M, AG, E, T	n.e., SD, SD, PD, SD
6	76	unk.	unk.	0/1	D, M, AG, T	SD, SD, PR, SD
7*	60	+	–	0/0	T, A, M	PR, SD, SD
8*	53	+	+	0/0	G followed by R, T, A	PR, SD, SD
9*	79	unk.	unk.	1/2	T, E, M, L/A,	SD, SD, SD, SD

Abbreviations: ER, estrogen receptor; PGR, progesterone receptor; ET, endocrine treatment; CT, chemotherapy; T, tamoxifen; M, megace; G, goserelin; F, formestane; F + AG, formestane and aminoglutethimide in concert; A, anastrozole; L, letrozole; L/A, letrozole–anastrozole crossover study; AG, aminoglutethimide; D, droloxifene; E, exemestane; R, radiation of the ovaries (castration); SD, stable disease; PR, partial response; CR, complete response; PD, progressive disease; *, participants in tracer-study (total body aromatization); n.e., not evaluable; unk., unknown.

[a] No. of chemotherapy regimens for advanced disease/total no. of regimens.