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Catecholestrogens excretion in smoking and non-smoking postmenopausal women receiving estrogen replacement therapy ☆

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Abstract

Estrogens are involved in the etiology of breast cancer. Their blastomogenic influence may be partly realized through their conversion into catecholestrogens, rate of which may be modified by smoking. The risk of having breast cancer diagnosed can increase in women using estrogen replacement therapy (ERT). The principal aim of this investigation was to compare the excretion of classical estrogens and catecholestrogens in smoking and non-smoking postmenopausal women receiving Progynova (estradiol valerate, 2 mg/day, 1 month). Total 16 women were studied before and after treatment. Urinary estrogen profile method based on isotope dilution capillary gas chromatography–mass spectrometry was used. Before ERT, significantly lower excretion of 16-epiestriol and 4-hydroxyestrone (4-OHE1) and lower ratio of 4-OHE1/E1 were revealed in smokers. After ERT, much higher excretion of 2-OHE1, and 4-hydroxyestradiol (4-OHE2), higher ratios of 2-OHE1/E1 and 4-OHE1/E1 and lower ratio of 2-methoxyestrone/2-OHE1 were discovered in smokers as compared to non-smoking women. In conclusion only combination of ERT + smoking and not smoking itself leads to the specific prevalence of catecholestrogens (2-OH- and carcinogenic and DNA-damaging 4-OH-metabolites) that may increase risk of genotoxic variant of hormone-induced breast carcinogenesis without influence on the total morbidity.

Keywords: Estrogens; Catecholestrogens; Smoking; Estrogen replacement therapy; Hormonal carcinogenesis

1. Introduction

Estrogens play an important role in the development and growth of cancer [1–3]. Studies conducted during recent years concluded that two main mechanisms or their combination may be involved in the process of estrogen-induced carcinogenesis i.e., increased mitogenesis or/and initial DNA-damage under the influence of estrogenic excess or prolonged estrogenic stimulation [4–6].

Other studies deal with the question in which form estrogens are involved in carcinogenesis and conclude that one of the important pathways of estrogen metabolism in this context is the conversion of estradiol to catecholestrogens [5,7,8]. Endogenous as well as exogenous estrogens may serve as a source for conversion into carcinogenic catecholestrogens [5,6]. In human subjects, however, involvement of estrogen-containing drugs into these biochemical reactions has been studied to a very limited extent.

According to the results of a large collaborative study, the risk of having breast cancer diagnosed is seem to increase in women using estrogen replacement

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therapy (ERT) by a factor of 1.023 for each year of use [9]. Though smoking does not influence this risk or only non-significantly increases it in cases of last use of ERT within 5 years before diagnosis [9], tobacco smoke, as was suggested and partly demonstrated by us [10,11], may promote shift from promotional to more tough genotoxic type of estrogen-induced carcinogenesis. Mechanism of such effect of combination of smoking with estrogens may be mediated through increased formation of catecholestrogens, some of which (so called 4-hydroxyestrogens) are considered as most carcinogenic and DNA-damaging among estrogen metabolites [5,12]. Previously with the use of a radiometric procedure, the stimulatory effect of cigarette smoking on estrogen 2-hydroxylation has been demonstrated in young women not receiving any treatment [13], though in a very limited number of women of reproductive age who smoked significant differences in mean values were not revealed compared with non-smokers [7].

Thus, the principal aim of the present investigation was to compare the excretion of classical estrogens and catecholestrogens in smoking and non-smoking postmenopausal women receiving ERT.

2. Materials and methods

2.1. Subjects

Sixteen women — 6 smokers and 10 non-smokers — to whom estradiol valerate (Progynova, 2 mg per os/day) because of modest degree vasomotor menopausal symptoms had been administered for 1 month, were studied before and next day after end of the treatment. Smokers were somewhat younger (51.8 ± 3.4 years, mean ± SD) than non-smokers (57.3 ± 4.0 years) although duration of menopause in these groups (respectively 4.2 ± 2.2 and 6.5 ± 3.6 years) and body weight (66.2 ± 125 and 69.2 ± 12.0 kg) were practically the same. Smokers consumed on average 15.7 ± 4.4 cigarettes/day during 28.0 ± 6.5 years.

2.2. Urine collection

Each of the sample periods included a complete 24-h collection of urine in plastic bottles without any special diet prescription. Urine samples were stored frozen with ascorbic acid (about 0.1–0.2%) at −20°C and when all samples were collected they were transported on dry ice to the laboratory in Helsinki.

2.3. Determination of estrogen fractions

Urinary estrogen profile method (15 estrogen metabolites) based on isotope dilution capillary gas chromatography–mass spectrometry was used. Details of the method are given elsewhere [7,14]. The following estrogen fractions were measured: estrone (E1), estradiol (E2), estriol (E3), 2-hydroxyestrone (2-OHE1), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestrone (4-OHE1), 4-hydroxyestradiol (4-OHE2), 2-methoxyestrone (2-MOE1), 2-methoxyestradiol (2-MOE2), 16-ketoestradiol (16ketoE2), 16α-hydroxyestrone (16α-OHE1), 16β-hydroxyestrone (16β-OHE1), 15α-hydroxyestrone (15α-OHEL), 16-epiestriol (16-epiE3) and 17-epiestriol (17-epiE3).

2.4. Statistical analysis

Group means ± standard deviations (SD) and some ratios for the estrogens were calculated. Means were compared with the *t*-test using the StatSoft Inc.,1993 program for Windows. In case of non-parametric evaluation of the data Mann–Whitney U-test and Wald–Wolfowitz runs test were used.

3. Results

Main findings of the study are presented in Tables 1 and 2. Before therapy with estradiol valerate significantly lower excretion of 16-epiE3 and 4-OHE1 (Table 1) and lower ratio of 4-OHE1/E1 (Table 2) were revealed in smokers compared with non-smoking postmenopausal women.

Treatment with Progynova modified excretion of all studied estrogen fractions although in a different degree in smoking and non-smoking women. After the course of estrogen replacement therapy, significantly higher excretion of 2-OHE1, 4-OHE2, 2-OHE1 + 2-OHE2 and 4-OHE1 + 4-OHE2, higher ratios 2-OHE1/E1, 4-OHE1/E1, 2-OHE1 + 2-OHE2/16α-OHE1 + E3 and lower ratio of 2-MOE1/2-OHE1 (Tables 1 and 2) were characteristic for smokers. There was a significantly higher increase (addition) in excretion of 2-OHE1 and 4-OHE2 (Table 1) and in sums 2-OHE1 + 2-OHE2 and 4-OHE1 + 4-OHE2 (data not shown) in smoking postmenopausal women as compared to non-smokers. The similar conclusions were received using non-parametric approach to the data evaluation (for example "*p*" value for the difference between excretion of 4-OHE2 in smoking and non-smoking women after ERT in Wald–Wolfowitz runs test was equal 0.036 and in Mann–Whitney U-test it was 0.014).

4. Discussion

According to epidemiologic evidence women who smoke cigarettes are relatively estrogen-deficient with

Table 1
Excretion of classical estrogens and other estrogen metabolites in studied postmenopausal women[a]

Estrogen[b]	Non-smokers			Smokers		
	Before treatment (A)	After treatment (B)	Value of (B − A)	Before treatment (A)	After treatment (B)	Value of (B − A)
E1	9.70 ± 8.60	1232.6 ± 295.2	1222.3 ± 292.5	14.46 ± 13.24	951.5 ± 181.2	937.0 ± 177.4
E2	2.87 ± 2.14	212.6 ± 42.2	209.7 ± 43.0	3.65 ± 2.60	235.7 ± 40.5	232.0 ± 42.7
E3	4.38 ± 2.37	178.3 ± 75.2	173.9 ± 75.6	3.31 ± 2.49	132.1 ± 83.2	128.8 ± 83.3
2-OHE1	8.88 ± 8.42	226.9 ± 68.3[c]	218.0 ± 73.6[c]	6.52 ± 3.78	330.9 ± 80.0[c]	324.4 ± 80.6[c]
2-OHE2	2.19 ± 2.09	36.4 ± 11.7	34.2 ± 12.6	2.05 ± 0.56	45.8 ± 9.2	43.8 ± 9.2
4-OHE1	2.29 ± 1.28[c]	28.8 ± 10.6	26.5 ± 11.1	1.09 ± 0.51[c]	43.7 ± 18.2	42.6 ± 18.4
4-OH2	0.07 ± 0.08	23.5 ± 15.6[c]	23.4 ± 15.7[c]	0.10 ± 0.13	60.8 ± 25.3[c]	60.7 ± 25.4[c]
2-MOE1	2.49 ± 1.57	53.4 ± 15.1	50.9 ± 15.1	2.36 ± 0.79	51.3 ± 21.9	48.9 ± 22.4
2-MOE2	0.37 ± 0.26	3.1 ± 0.8	2.7 ± 0.7	0.41 ± 0.45	3.2 ± 1.1	2.9 ± 1.2
16 KetoE2	2.49 ± 1.91	66.2 ± 33.1	63.7 ± 33.4	1.28 ± 0.86	54.7 ± 42.7	53.4 ± 43.4
16α-OHE1	3.79 ± 3.40	104.8 ± 78.1	101.0 ± 77.3	2.46 ± 1.40	105.8 ± 66.8	103.3 ± 67.2
16β OHE1	6.90 ± 7.17	50.8 ± 20.4	43.9 ± 18.4	4.60 ± 1.27	39.9 ± 21.1	35.3 ± 22.3
15α OHE1	0.46 ± 0.86	15.0 ± 8.5	14.5 ± 8.7	0.22 ± 0.30	11.2 ± 3.7	11.0 ± 3.7
16-epiE3	1.12 ± 0.62[c]	24.2 ± 9.0	23.1 ± 9.2	0.47 ± 0.28[c]	12.5 ± 15.1	12.0 ± 15.1
17-epiE3	0.60 ± 0.20	2.8 ± 2.7	2.2 ± 2.7	0.49 ± 0.26	1.8 ± 1.2	1.3 ± 1.2

[a] Means and standard deviations (nmol/24 h).
[b] See text for abbreviations.
[c] Difference between smokers and non-smokers is significant (p at least < 0.05).

an increased risk of osteoporosis and a decreased risk of endometrial [15] and — only in certain groups [16] — breast cancer. Data on estrogen levels have not been consistent and for example, both circulating and urinary classic estrogens have not been found to differ among smoking and non-smoking postmenopausal women [15,17] with only some exclusions [18]. Though a pharmacokinetic study showed significantly lower levels of unbound estradiol in women who smoke than in non-smokers after 1–2 mg of oral micronized estradiol, smoking had no apparent influence on circulating estrogens concentrations in postmenopausal women after treatment with percutaneous form of this estrogen [19].

Conflicting opinions were presented regarding question which pathway of estrogen metabolism is mainly implicated into estrogen-induced carcinogenesis [1,7,8,20] and the role of catecholestrogens and products of their metabolism in this process has been recently repeatedly emphasized [5–8]. Data obtained in the present investigation demonstrate no difference in excretion of classic estrogens (E1, E2, E3) before and after ERT between smoking and non-smoking postmenopausal women (Tables 1 and

Table 2
Ratios and sums of urinary estrogen metabolites in studied postmenopausal women[a]

Estrogen[b]	Non-smokers		Smokers	
	Before treatment	After treatment	Before treatment	After treatment
E1 + E2 + E3	16.95 ± 12.97	1623 ± 358.6	21.42 ± 16.15	1319. ± 243.1
2-OHE1 + 2-OHE2	11.07 ± 10.50	263.3 ± 78.3[c]	8.57 ± 3.84	376.7 ± 84.4[c]
4-OHE1 + 4-OHE2	2.36 ± 1.29	52.3 ± 25.3[c]	1.19 ± 0.56	104.5 ± 43.4[c]
2-OH1/E1	0.95 ± 0.45	0.19 ± 0.07[c]	0.57 ± 0.21	0.35 ± 0.09[c]
2-OHE2/E2	0.69 ± 0.25	0.17 ± 0.06	0.86 ± 0.57	0.20 ± 0.06
4-OHE1/E1	0.33 ± 0.25[c]	0.03 ± 0.01[c]	0.11. ± 0.05[c]	0.05 ± 0.01[c]
2-OHE1/4-OHE1	3.91 ± 2.52	8.32 ± 2.32	5.81 ± 1.54	7.96 ± 1.54
2-MOE1/2-OHE1	0.37 ± 0.21	0.24 ± 0.07[c]	0.44 ± 0.19	0.15 ± 0.03[c]
E3/E1 + E2	041 ± 0.13	0.12 ± 0.05	0.27 ± 0.16	0.10 ± 0.06
16α-OHE1/E1	0.41 ± 0.20	0.08 ± 0.05	0.31 ± 0.31	0.11 ± 0.06
16β-OHE1/E1	0.80 ± 0.42	0.04 ± 0.01	0.54 ± 0.37	0.04 ± 0.02
2-OHE1 + 2-OHE2/16α-OHE1 + E3	1.23 ± 0.32	1.08 ± 0.45[c]	1.62 ± 0.45[c]	2.25 ± 1.53[c]

[a] Means and standard deviations.
[b] See text for abbreviations.
[c] Difference between smokers and non-smokers is significant (p at least < 0.05).

2). Lower excretion of 4-OHE1 was characteristic for smokers before start of ERT. At the same time, a higher excretion of 2- and 4-hydroxyestrogens (absolute and relative values) was revealed in smoking postmenopausal women (compared with non-smokers) after a course of estrogen treatment (Tables 1 and 2).

Although rather controversial views were presented with regard to possible problastomogenic activity of 2-hydroxyestrogens [7,20,21], these estrogen metabolites prevent inactivation of 4-hydroxyestrogens mediated by catechol-*O*-methyltransferase [8]. In their turn 4-hydroxyestrogens are considered (see above) as powerful carcinogens possessing hormonal and DNA-damaging activity [5,12]. Thus, increase in production of 4-hydroxyestrogens in smokers receiving ERT can be instrumental in shift from the promotional to genotoxic type of hormone-induced carcinogenesis characteristic for smoking as previously suggested [10,11,17]). This variant of hormonal carcinogenesis may be more tough clinically which partly explains why course of endometrial or breast cancer is more fast and prognosis worse though frequency of disease is respectively lower or not changed in smokers compared with non-smoking women [22,23]. If one recollects "defensive" (antiangiogenic and antitumor) properties of 2-methoxyestrogens [24,25], then decrease in the ratio 2-MOE1/E1 revealed in this study in postmenopausal smokers receiving estrogen (Table 2) will provide a general picture with an important additional feature. On the other side, the local activities of some enzymes of estrogen metabolism (aromatase, sulfatase, estrogenic 2- and 4-hydroxylases and others) may be more relevant for some target tissues [2,5,26] and need to be taken into consideration too.

In conclusion, only combination of smoking + ERT and not smoking itself leads to the specific prevalence of catecholestrogens (2-hydroxy- and carcinogenic and DNA-damaging 4-hydroxymetabolites) that may increase risk of genotoxic variant of hormone-induced carcinogenesis. As the present investigation was restricted to 1 month estrogen treatment and as such treatment became rather popular in several conditions [27] more prolonged studies of ERT effect on estrogen metabolism and catecholestrogens production in smokers need to be conducted.

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