Excretion of Catecholestrogens in Women Receiving Estrogen-Substitution Therapy

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We compared excretion of classical estrogens and catecholestrogens in smoker and nonsmoker postmenopausal women receiving estrogen-substitution therapy. Combined effect of substitution therapy and smoking, but not smoking along increased production of catecholestrogens, which could enhance the risk of genotoxic (clinically more severe) variant of hormone-induced carcinogenesis.

Key Words: *estrogens; catecholestrogens; smoking; menopause; carcinogenesis*

Estrogens play an important role in the development of a number of diseases including malignant tumors [2,7]. Recent studies revealed two basic mechanisms of hormonal carcinogenesis associated with a surplus of estrogens and/or their metabolites: intensification of mitogenesis (promotor type) and/or primary damage of DNA (genotoxic type) [1,8,9]. One of the important metabolic pathways of classical estrogens is their conversion into catecholestrogens [3]; the effect of estrogen-containing drugs on this process is little studied. However, numerous studies showed that risk of breast tumors increases 1.023-fold every year in women receiving estrogen-substitution therapy (EST) [5]. Although smoking virtually does not affect this risk, it was suggested that the components of smoke can switch the type of estrogen-provoked carcinogenesis from the promotor to more unfavorable genotoxic type [1]. The detailed mechanism of such combined effect of smoking and estrogens and the role of catecholestrogens in this process are not clear. Some of catecholestrogens (4-hydroxyestrogens) are considered the most potent carcinogenic agents among all estrogen metabolites capable of damaging DNA [11].

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Our aim was to compare excretion of classical estrogens and catecholestrogens in smoker and nonsmoker postmenopausal women receiving EST.

MATERIALS AND METHODS

We examined 16 women (6 smokers and 10 nonsmokers) with moderate vasomotor symptoms accompanying menopause receiving estradiol valerate (progynova, 2 mg/day) during one month. Smoker women were slightly younger than nonsmokers (51.0±3.4 and 57.3±4.0 years, respectively). Duration of menopause (4.2±2.2 and 6.5±3.6 years, respectively) and body weight (66.2±12.5 and 69.2±12.0 kg, respectively) were virtually the same in both groups. The mean intensity of smoking was 15.7±4.4 cigarettes per day during 28.0±6.5 years. Before treatment and on the next day after cessation, 24-h urine was collected in plastic flasks and ascorbic acid (0.1-0.2%) was added. Urine samples were stored at -20°C and transferred to the laboratory on dry ice.

The spectrum of urine estrogens (15 metabolites) was determined by capillary gas chromatography and mass-spectrometry [3]. The following fractions of estrogens were determined: estrone (E1), estradiol (E2), estriol (E3), 2-hydroxyestrone (2-OH-E1), 2-hydroxyestradiol (2-OH-E2), 4-hydroxyestrone (4-OH-E1), 4-hydroxyestradiol (4-OH-E2), 2-methoxyestrone

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(2-MO-E1), 2-methoxyestradiol (2-MO-E2), 16-ketoestradiol (16-ketoE2), 16α-hydroxyestrone (16α-OH-E1), 16β-hydroxyestrone (16β-OH-E1), 15α-hydroxyestrone (15α-OH-E1), 16-epiestriol (16-epiE3), and 17-epiestriol (17-epiE3). The means and standard deviations were calculated, the data were statistically analyzed using Student's *t* test.

RESULTS

Before estradiol therapy smokers had markedly decreased excretion of 16-epiE3 and 4-OH-E1 (Table 1) and lower 4-OH-E1/E1 ratio compared to nonsmoker women (Table 2). EST changed excretion of all measured estrogen fractions, but the degree of these changes in smokers and nonsmokers was different. After EST, excretion of 2-OH-E1, 4-OH-E2, the sum of 2-OH-E1+2-OH-E2 and 4-OH-E1+4-OH-E2 in smoker patients markedly increased, the ratios of 2-OH-E1/E1, 4-OH-E1/E1, and 2-OH-E1+2-OH-E2/16α-OH-E1+E3 increased, while the ratio of 2-MO-E1/2-OH-E1 decreased (Tables 1 and 2). The excretion of 2-OH-E1 and 4-OH-E2 (Table 1) and the sum of 2-OH-E1+2-OH-E2 and 4-OH-E1+4-OH-E2 more markedly increased in smokers, than in nonsmokers.

Blood and urine concentrations of classical estrogens in postmenopausal smoker and nonsmokers women do not differ significantly [4], although previous studies demonstrated enhanced excretion of 2-hydroxyestrogens in young healthy smoker women [10]. There is no evidence on the effect of exogenous estrogens on the dynamics of these indices. We revealed no differences in the excretion of classical estrogens (E1, E2, E3) between smoker and nonsmoker women before and after EST (Tables 1 and 2). Although excretion of 4-OH-E1 in smokers receiving no EST was moderated, excretion of 2- and 4-hydroxyestrogens significantly increased in these patients after the course of EST (Tables 1 and 2). There is evidence that 2-hydroxy estrogen derivatives prevent inactivation (methoxylation) of 4-catecholestrogens catalyzed by catechol-O-methyl transferase [12]. In turn, 4-hydroxyestrogens are very potent carcinogens exhibiting hormonal activity and damaging DNA [11]. Therefore, the enhanced production of 4-hydroxyestrogens in smokers receiving EST could be a factor provoking "switch" of hormone-induced carcinogenesis from the promotor type to genotoxic one. This partially explains more rapid development and less favorable prognosis of carcinoma in the endometrium or breast cancer in smoker women compared to nonsmokers [6]. Taking into consideration the antitumor activity of 2-methoxyestrogens [12] revealed in this work, the decrease in 2-MO-E1/E1 ratio in smoker postmenopausal women seems to be important.

In conclusion, it should be noted that only combination of smoking and EST, but not smoking along determines the prevalence of catecholestrogens (2-hydroxy metabolites, carcinogenic and DNA-damaging 4-hydroxy metabolites), which may enhance the risk

TABLE 1. Excretion of Classical Estrogens and Their Metabolites (nM/day) in Postmenopausal Women (*M±m*)

Estrogen	Nonsmokers		Smokers	
	before therapy	after therapy	before therapy	after therapy
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±42.7 (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-OH-E1	8.88±8.42	226.9±68.3 (218.0±73.6)	6.52±3.78	330.9±80.0* (324.4±80.6*)
2-OH-E2	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-OH-E1	2.29±1.28	28.8±10.6 (26.5±11.1)	1.09±0.51*	43.7±18.2 (42.6±18.4)
4-OH-E2	0.07±0.08	23.5±15.6 (23.4±15.7)	0.10±0.13	60.8±25.3* (60.7±25.4*)
2-MO-E1	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-MO-E2	0.37±0.26	3.1±0.8 (2.7±0.7)	0.41±0.45	3.2±1.1 (2.8±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-OH-E1	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-OH-E1	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-OH-E1	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	24.2±9.0 (23.1±9.2)	0.47±0.28*	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)

Note. The difference of indices before and after treatment is given in parentheses. Here and in Table 2: *$p<0.05$ compared to nonsmokers.

TABLE 2. Relationships and Total Content of Different Estrogen Metabolites (*M*±*m*)

Estrogen	Nonsmokers		Smokers	
	before treatment	after treatment	before treatment	after treatment
E1+E2+E3	16.95±12.97	1623.5±358.6	21.42±16.15	1319.3±243.1
2-OH-E1+2-OH-E2	11.07±10.50	263.3±78.3	8.57±3.84	376.7±84.4*
4-OH-E1+4-OH-E2	2.36±1.29	52.3±25.3	1.19±0.56	104.5±43.4*
2-OH-E1/E1	0.95±0.45	0.19±0.07	0.57±0.21	0.35±0.09*
2-OH-E2/E2	0.69±0.25	0.17±0.06	0.86±0.57	0.20±0.06
4-OH-E1/E1	0.33±0.25	0.03±0.01	0.11±0.05*	0.05±0.01*
2-OH-E1/4-OH-E1	3.91±2.52	8.32±2.32	5.81±1.54	7.96±1.54
2-MO-E1/2-OH-E1	0.37±0.21	0.24±0.07	0.44±0.19	0.15±0.03*
E3/E1+E2	0.41±0.13	0.12±0.05	0.27±0.16	0.10±0.06
16α-OH-E1/E1	0.41±0.20	0.08±0.05	0.31±0.31	0.11±0.06
16β-OH-E1/E1	0.80±0.42	0.04±0.01	0.54±0.37	0.04±0.02
2-OH-E1+2-OH-E2/16α-OH-E1+E3	1.23±0.32	1.08±0.45	1.62±1.05	2.25±1.53*

of hormonal genotoxic carcinogenesis. Further studies of the effects of long-term EST on estrogen metabolism are needed.

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