ONCOLOGY

Enzymes of Estrogen Metabolism in Endometrial Cancer

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Activities of estrogen metabolism enzymes (aromatase, 2- and 4-estrogen hydroxylases, catechol-O-methyltransferase, and glutathione transferase) were studied by modern biochemical methods in tumors of patients with endometrial cancer. Relationships between enzyme activities and body weight index, age of menarche, stage of the disease, tumor histotype, differentiation degree, and depth of invasion into the myometrium were detected. The detected relationships between enzyme activities and serum concentrations of estradiol and progesterone and level of estrogen receptors in tumor tissue attest to hormone dependence of aromatase, estrogen hydroxylases, and glutathione transferase.

Key Words: *estrogen metabolism enzymes; endometrial cancer*

Estrogens in the majority of cases are essential for the development of hyperplastic processes and for initiation and maintenance of malignant tumor growth in the endometrium [2,3]. In the endometrium estrogens are synthesized from androgens under the effect of aromatase. In genotoxic type of hormonal carcinogenesis estrogens are transformed into catecholestrogens with participation of 2- and 4-estrogen hydroxylases. Further metabolism of catecholestrogens is catalyzed by catechol-O-methyltransferase (COMT) and glutathione transferase (GT) and yields inert and nonmutagenic derivatives. If these enzyme systems are ineffective, catecholestrogen ortho-quinones are formed under the effect of peroxidases or via non-enzymatic pathways. Catecholestrogen ortho-quinones can initiate tumor growth when DNA damage induced by them involve oncogenes or suppressor genes [6]. In case of hyperplastic processes and cancer of the endometrium, primarily promotor type of hormonal carcinogenesis associated with activation of estrogen receptors is discussed [5]. The role of enzymes of estrogen metabolism in genotoxic carcinogenesis was confirmed on several experimental models, on clinical model of breast cancer, and was virtually not studied in endometrial cancer [6].

We studied activity of enzymes of estrogen metabolism in endometrial tumors and correlation of this parameter with clinical and morphological values, hormonal changes, and receptor status of the tumor.

MATERIALS AND METHODS

The study included 45 patients with endometrial cancer (mean age 57±2 years). In accordance with TNM and FIGO international classifications, 8 patients presented with *Ca in situ* cancer, 26 with stage I, and 11 with stage II cancer. Clinical and morphological examination included evaluation of patient age, menstrual and reproductive status, stage of the disease, histological type of the tumor, degree of differentiation, and depth of invasion in the

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myometrium, and estimation of body weight index. Morphological analysis revealed endometrioid carinomas of different degree of differentiation in 38 patients and nonendometrioid tumors in 7.

Serum levels of estradiol, progesterone, and sex steroid-binding globulin were measured using Immunotech radioimmunoassay kits. Index of free estrogens was calculated by the formula: index of free estrogens=estradiol (nmol/liter)×100/sex steroid-binding globulin (nmol/liter). Enzyme activities and levels of receptors were measured in samples obtained during surgery. Aromatase activity was evaluated by the formation of heavy water from 1β-tritiated androstenedione. Radioactivity was counted on the tritium channel of MICROBETA liquid scintillation counter (Perkin Elmer). Summary $^2/_4$-estrogen-hydroxylase activity was evaluated by radio enzyme assay [7]. COMT activity in the endometrium was evaluated by radiometry [8]. Activity of GT was evaluated spectrophotometrically by the formation of chromogenic conjugates of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) [4]. Protein was measured by the method of Lowry. The levels of estrogen receptors and progesterone in endometrial tumors were evaluated using traditional radioligand method [1]. [2,4,6,7-^{3}H]-estradiol and [1,2,6,7-^{3}H]-progesterone (Amersham Biosciences) were used.

The results were processed using Mann—Whitney nonparametric test. Spearman rank correlation coefficient was calculated. The differences were significant at $p<0.05$.

RESULTS

Specific features in enzyme activities depending on the stage of the disease were detected. The minimum estrogen hydroxylase and COMT activities were observed in *Ca in situ*. Maximum activity of estrogen hydroxylase was detected in stage II and of COMT in stage I endometrial cancer. Summary $^2/_4$-estrogen hydroxylase activity in endometrial *in situ* cancer differed significantly from its values in disease stages I and II. Aromatase and GT activities in *Ca in situ* were high and decreased with increasing the depth of tumor invasion into the myometrium. Dissemination of the tumor to the neck of the uterus was associated with an increase in aromatase and GT activities. However, no significant differences in enzyme activities in different stages of the disease were detected.

Enzyme activities were measured in tumors of different differentiation degree (Table 1). Statistically significant differences were detected only for COMT. The difference in COMT activity in well- and moderately-differentiated endometrial tumors was significant ($p<0.01$). None of poorly-differentiated tumors was aromatase-negative, while some well- and moderately-differentiated tumors were aromatase-negative. On the other hand, activities of COMT and GT were zero in 9 and 18% poorly-differentiated adenocarcinomas, respectively, which can indicate accumulation of highly aggressive intermediates stimulating high biological aggressiveness of these tumors.

TABLE 1. Activities of Aromatase (fmol Androstenedione/mg protein/h), Summary $^2/_4$-Estrogen Hydroxylase Activity (pmol methoxyestrogen/mg protein/h), Activities of COMT (nmol guaiacol/mg protein/h), and GT (μmol CDNB-SG/mg protein/min) in Endometrial Cancer Tissue Depending on Clinical and Morphological Parameters of Tumors

Parameter	Median values (25-75%)			
	aromatase	estrogen hydroxylases	COMT	GT
Stage				
"*Ca in situ*" (*n*=8)	26.0 (9.4-34.0)	11.8 (2-12)	81.0 (26.1-228.0)	288 (12.3-396.0)
I (*n*=26)	12.2 (6.6-22.5)	29.8 (15.8-95.5)*	287 (126-457)*	105 (50-230)
II (*n*=11)	14.7 (8.4-50.0)^o	35.6 (26.7-37.1)*$^+$	188 (59-397)*$^+$	273 (83-783)
Differentiation degree				
G1 (*n*=7)	19.2 (9.4-33.5)	24.5 (10.4-38.5)	183 (81-228)	121 (68-164)
G2 (*n*=19)	12.6 (5.4-21.8)	31.8 (13.90-53.55)	314 (258-365)^{ox}	195 (66-378)
G3 (*n*=12)	13.6 (8-34)	24.4 (12.6-29.1)	98.2 (25-397)^{ox}	225 (64-466)
Histological type				
endometrioid (*n*=38)	14.5 (7.4-30.0)	38.3 (11.8-38.1)	228 (126-327)	128 (66-367)
nonendometrioid (*n*=7)	23.9 (9.8-32.3)	110 (40-173)^a	278 (124-678)	118 (209-239)

Note. $p<0.05$ compared to: **Ca in situ*, $^+$I; $p<0.01$ compared to: ^{o}G1, ^{x}G2; $^a p<0.05$ compared to endometrioid type.

Activities of enzymes of estrogen metabolism were analyzed in tumors of different histological types. Estrogen hydroxylase activities in nonendometrioid tumors were significantly higher than in endometrioid tumors ($p<0.05$). None of nonendometrioid tumors was aromatase-positive, while 8% endometrioid carcinomas were aromatase-positive.

Analysis of correlations was carried out in order to evaluate the relationships between enzyme activities and clinical, morphological, and hormonal parameters and level of steroid hormone receptors. Relationships between aromatase activity and level of estrogen receptors ($r=0.309$; $p<0.05$) and between aromatase and GT activities ($r=0.399$; $p<0.05$) were detected. Estrogen hydroxylase activities in endometrial tumors correlated with serum estradiol and progesterone levels ($r=-0.582$ and $r=-0.601$; $p<0.05$) and with index free estrogens and level of estrogen receptors ($r=-0.645$ and $r=-0.533$; $p<0.05$). Activity of COMT correlated with body weight index ($r=0.577$; $p<0.05$), while GT activity correlated with the age of menarche, serum progesterone concentration, depth of tumor invasion into the myometrium, and level of estrogen receptors ($r_1=0.466$, $r_2=-0.326$, $r_3=-0.373$, and $r_4=-0.333$; $p<0.05$).

Hence, activities of enzymes of estrogen metabolism in endometrial tumors correlate with such clinical and morphological parameters as stage of the disease and depth of tumor invasion into the myometrium, and serum concentrations of estradiol and progesterone and level of estrogen receptors. Enzyme activities depended on the stage of tumor process. Activities of estrogen hydroxylases and COMT were different in pre-invasive and invasive cancer. In addition, a negative relationship between GT and depth of tumor invasion into the myometrium was detected. It seems that 2- and 4-estrogen hydroxylases, COMT, and GT are essential for tumor invasion into the myometrium. The detected relationships between enzyme activities and serum estradiol and progesterone levels and level of estrogen receptors in tumor tissue indicate a certain hormone dependence of aromatase, estrogen hydroxylases, and GT.

REFERENCES

1. L. S. Bassalyk, E. S. Gerstein, N. E. Kushlinskii, *et al.*, *Detection of Steroid Hormone Receptors in Hormone-Dependent Tumors and Prospective Endocrine Therapy (Methodological Recommendations)* [in Russian], Moscow (1987).
2. L. M. Berstein, *Hormonal Carcinogenesis* [in Russian], St. Petersburg (2000).
3. L. M. Berstein, A. E. Chernobrovkina, V. B. Gamayunova, *et al.*, *Vopr. Onkol.*, **49**, No. 1, 55-59 (2003).
4. V. Z. Lankin, A. K. Takhaze, and A. L. Koval'skaya, *Dokl. Akad. Nauk SSSR*, **261**, No. 6, 1467-1470 (1981).
5. Z. Sh. Gilyazutdinova, M. K. Mikhailov, Eds., *Oncology: Manual for Physicians* [in Russian], Moscow (2000).
6. E. L. Cavalieri, E. C. Rogan, and D. Chakravarti, *Cell. Mol. Life Sci.*, **59**, 665-681 (2002).
7. C. N. Theron, V. A. Russel, and J. J. Taljard, *J. Steroid Biochem.*, **25**, No. 4, 585-591 (1986).
8. G. Zurcher and M. Da Prada, *J. Neurochem.*, **38**, No. 1, 191-195 (1982).