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Aromatase and comparative response to its inhibitors in two types of endometrial cancer☆

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Abstract

Aromatase activity (AA) was evaluated totally in 80 tumors collected from primary endometrial cancer (EC) patients. All patients were divided into cases belonging to the types I or II of EC (respectively, 50 and 30 observations). Samples of malignant endometrium from type II demonstrated inclination to the higher AA in comparison with type I samples; the difference reached level of statistical significance in non-smoking patients ($p = 0.02$). Although no positive correlation was revealed between AA in EC tissue and percentage of cells with DNA damage in normal endometrium from the same patients, the rate of DNA damage (percent of comets, comet's tail average length, etc.) was higher in intact endometrium collected from patients with type II of the disease. In 19 tumor samples, CYP19 gene expression was evaluated by RT-PCR and level of mRNA signal demonstrated positive correlation with AA ($R_s = +0.63$, $p = 0.05$) in the whole this material. Of note, though, CYP19 mRNA expression was not revealed in six cases, and all of them belonged to the type I of disease. Finally, in 23 EC patients (15 with type I and 8 with type II of the disease) effects of 2 weeks treatment with letrozole (10 pts) and exemestane (13 pts) were evaluated in neoadjuvant setting. Although diminishing of endometrial M-echo signal and the increases in FSH and LH concentration after treatment were more pronounced in type I patients, decrease in tumor PR content ($p = 0.04$) was more revealing in patients with type II of EC; besides, the decreases in AA in tumor tissue by the end of treatment were noted predominantly in patients with lower body weight (BMI <27.5). Thus, although type II of EC is frequently considered as hormone-independent, increased ability of this type of the tumor to estrogen biosynthesis (at CYP19 gene and protein level) may lead to the reconsideration of such conclusion and warrants further investigation. The search of possible ethnic differences in AA and in the biologic response to aromatase inhibitors in EC can be of importance too.

Keywords: Aromatase; CYP19; Activity; Expression; Aromatase inhibitors; Endometrial cancer; Types

1. Introduction

Intratumoral estrogen biosynthesis may be considered as a reserve in understanding of the mechanisms of endometrial cancer (EC) development and progression and in elaboration of measures for the increase in efficiency of its treatment. Although no completely deprived of controversy [6], the leading contemporary concept claims that normal human endometrium does not contain aromatase mRNA transcripts, and ability to synthesize estrogens is characteristic only for endometrial cancer itself [7,12]. Meanwhile, aromatase activity (AA) in EC tissue has been evaluated much rarely than in breast cancer, and to our knowledge practically no comparative studies have been performed relating levels of aromatase activity and CYP19 mRNA within malignant endometrium [13].

The important feature of EC is its heterogeneity. Several lines of evidence suggest that two different variants of EC can be distinguished [1,5,8,14]. One of these variants is traditionally called hormone-dependent or estrogen-dependent and is designated as type I. Another variant is usually considered hormone-independent or estrogen-independent and called otherwise as type II. Each of these types is

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characterized by rather specific complex of pathological, genetic and clinical features of the tumor as well as by a set of typical characteristics of the host [5,8,14]. The principal aim of present study was to compare AA and CYP19 gene expression in tumor tissue in cases belonging to the types I or II of endometrial cancer, as well as to evaluate response of such patients and their neoplasms to the short-term pre-operational treatment with aromatase inhibitors.

2. Materials and methods

2.1. Patients

Tumor samples from 80 patients with EC were evaluated. Age of the patients varied between 38 and 80, and 72 patients were postmenopausal. According to existing surgical classification, stage of the disease varied between FIGO IA-IIIC. Morphologically mostly (approximately in 90% of cases) endometrioid adenocarcinomas were presented in this material. To distinguish between patients with types I and II of EC mainly the following criteria were used: absence or presence of infertility/low pregnancy number, late menopause, obesity, hypertension, hyperlipidemia, uterine myoma, endometriosis, ovarian theca hypertrophy, well-, modestly- or low-differentiated carcinomas, superficial (less than 5 mm) or deep invasion into myometrium, involvement of regional lymph nodes. As proposed, patients who are infertile, obese, hypertensive, hyperlipidemic, have menopause timing $\geq$53 years, myoma or endometriosis and well-differentiated node-negative adenocarcinoma with superficial invasion (or are characterized with more than 60% of these features) belong to the type I of disease [5,8]. As a result, it was considered that among studied group of patients 50 women had type I of EC and 30 women type II. In 23 of 80 aforementioned EC patients (15 with type I and 8 with type II of the disease), after the tissue specimens for aromatase activity determination were received during diagnostic biopsy, effects of 2 weeks treatment with aromatase inhibitors letrozole (2.5 mg/day, 10 pts) and exemestane (25 mg/day, 13 pts) were evaluated in neoadjuvant setting.

2.2. Experimental procedures

Aromatase activity in tumor tissue was estimated by measuring of ^3H_2O release from ^{3}H-1-β-androstenedione (NEN, Boston, MA; specific activity, 25.4 Ci/mM) as described [15]. Briefly, the reaction mixture (which contained tumor homogenate, NADPH regeneration system and labeled androgenic precursor) was incubated for 2 h at 37 °C. Then reaction was stopped by adding 5 vol of cold chloroform and 5% suspension of activated charcoal (Norit A) was added to the water phase. The fraction containing ^3H_2O was separated by centrifugation, and counting was performed with dioxane scintillator. Results were presented in fM/mg protein/h.

CYP19 gene expression was studied in 19 samples of malignant endometrium, in which AA has been measured as well. Total RNA was isolated with guanidinium thiocyanate – phenol – chloroform method and checked by spectrophotometry and electrophoresis. Reverse transcription was performed in standard way and its efficiency was verified with PCR with primers to GAPDH-gene. Expression of the coding site of aromatase mRNA was evaluated examining the region across exons II and III with primers described by Koos et al. [9]—sense: 5′-GAATATTGGAAGGATGCACAGACT-3′, antisense: GGGTAAAGATCATTTCCAGCATGT-3′. The conditions of reaction were the following: 37 cycles, denaturation—30 s at 95°, annealing—30 s at 61°, synthesis—1.5 min at 72°. The products were separated in the polyacrylamide gel and visualized by ethidium bromide staining. The expected size of CYP19 product was 293 bp and as a positive control RNA from human placenta or breast cancer was used. Results were presented in a semiquantitative manner using arbitrary or conditional units: 1 ("−" negative signal), 2 ("±" weak positive signal) and 3 ("+" strong positive signal).

In 12 primary patients (6 with type I of EC and another 6 with type II), samples of normal endometrium were collected simultaneously with cancer specimens with the aim to compare aromatase activity in the tumor with DNA damage rate in macroscopically normal endometrial tissue. DNA damage was evaluated on the basis of COMET assay in the modification suitable for cells isolated from solid tissues [17].

Effect of neoadjuvant treatment of 23 EC patients with aromatase inhibitors (see above, Section 2.1) has been determined. For that, clinical, sonographic, morphologic, cytologic and hormonal-metabolic parameters (blood estradiol, LH, FSH, glucose and cholesterol levels; tumor aromatase activity and steroid hormone receptors (ER and PR) content by methods used in our laboratory [4]) were evaluated before and after the course.

Statistical analysis was performed by methods allowing for means, standard errors, χ^2-values and Spearman correlation on the basis of SigmaPlot program. The differences with $p \leq 0.05$ were considered as significant.

3. Results

AA varied in the specimens of malignant endometrium between 0 and 28.4 fM/mg protein/h; in 43.1% of samples it was 5 fM/mg protein/h and less and in 16.3% higher than 15 fM/mg protein/h. Average value of AA in tumor specimens from patients belonging to the type II of EC was higher in comparison with type I cases; this difference reached the level of statistical significance in non-smoking patients (Table 1). Interestingly, prevalence of the specimens with AA value $\leq$5 fM/mg protein/h has been in patients with type II of EC significantly lower (27 ± 8%) than in type I cohort (54 ± 7%, $p = 0.02$).