A NEW STRATEGY IN SELECTION OF HORMONE THERAPY FOR ENDOMETRIAL PROLIFERATIVE PROCESS IN POSTMENOPAUSAL PATIENTS

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The limited efficacy of hormone therapy for endometrial proliferative process (EPP) in postmenopausal patients and its side effects on the immune system functionalities have not been studied in detail. Here we assess the feasibility of hormone therapy for EPP in postmenopausal patients through evaluation of estradiol and progesterone receptor gene expression in endometrial tissue and peripheral blood mononuclear cells (PBMC). The study enrolled 92 postmenopausal patients with EPP, including 37 pts with glandular-fibrous polyps, 7 pts with non-atypical endometrial hyperplasia (EH), 8 pts with atypical endometrial hyperplasia (AEH), 31 pts with moderately differentiated adenocarcinoma and 9 pts with highly differentiated adenocarcinoma. The PBMC isolates and endometrial samples were tested for ERα, ERβ, mER, PRA, PRB, mPR and PGRmC1 expression by reverse real time polymerase chain reaction (RT–PCR). Differential changes in PBMC receptor profiles upon in vitro exposure to progesterone or mifepristone were determined for patients with endometrial polyps and healthy women. The results indicate elevated expression of ERα, ERβ, PRA, PRB, mPR and PGRmC1 by endometrial tissues in EH and elevated expression of mER, ERα and PRA by PBMC in AEH, apparently reflecting suppressed functionalities of monocytes, macrophages, T-cells and natural killer cells. Unaltered expression of the studied genes by PBMC in endometrial adenocarcinoma may reflect the incrementing tumor autonomy. In vitro, mifepristone inhibited ERα, ERβ, mPR, PGRmC1, PRA and PRB expression in PBMC isolated from patients with endometrial polyps. We suppose that such effects can mitigate the negative influence of sex steroid hormones on immunocompetent cells.

Keywords: endometrial proliferative process, endometrial hyperplasia, postmenopause, hormonal therapy, ERα, ERβ, mER, PRA, PRB, mPR, PGRmC1

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Compliance with ethical standards: the study was approved by Ethical Review Board at the Pirogov Russian National Research Medical University (Protocol № 210 of 30 August 2021). All patients provided voluntary informed consent for the study.

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НОВАЯ СТРАТЕГИЯ ПОИСКА ГОРМОНАЛЬНОЙ ТЕРАПИИ ПРОЛИФЕРАТИВНЫХ ПРОЦЕССОВ ЭНДОМЕТРИЯ У ПАЦИЕНТОК В ПОСТМЕНОПАУЗЕ

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Причины неэффективности гормональной терапии пролиферативных процессов эндометрия (ППЭ) в постменопаузе остаются неясными. Влияние гормональной терапии ППЭ на активность иммунной системы достаточно не изучено. Целью работы было на основании результатов исследования экспрессии генов рецепторов эстрадиола и прогестерона в ткани эндометрия, клетках мононуклеарной фракции периферической крови (МНФК) определить целесообразность и возможность назначения гормональной терапии ППЭ у пациенток в постменопаузе. Обследовали 92 пациентки в постменопаузе с ППЭ: с железисто-фиброзными полипами — 37; с гиперплазией без атипии (ГЭ) — 7; атипической гиперплазией (АГЭ) — 8; умереннодифференцированной аденокарциномой — 31; высокодифференцированной аденокарциномой — 9. В МНФК и образцах ткани эндометрия исследовали экспрессию ERα, ERß, mER, PRA, PRB, mPR, PGRmC1 методом РТ-ПЦР. В эксперименте определяли изменение рецепторного профиля МНФК после инкубации клеток с прогестероном и мифепристоном у пациенток с полипами эндометрия и здоровых женщин. При анализе результатов в ткани ГЭ обнаружен высокий уровень ERα, ERβ, PRA, PRB, mPR, PGRmC1. В МНФК при АГЭ выявлен высокий уровень mER, ERα, PRA. Это может свидетельствовать о подавлении функции моноцитов, макрофагов, Т-лимфоцитов и натуральных киллеров. При аденокарциноме эндометрия рецепторный транскриптом в МНФК не нарушен, что может означать развитие автономности опухоли. В исследовании *in vitro* мифепристон снижает экспрессию ERα, ERß, mPR, PGRmC1, PRA, PRB в МНФК у пациенток с полипами эндометрия. На наш взгляд, это нивелирует негативное действие половых стероидных гормонов на иммунокомпетентные клетки.

Ключевые слова: пролиферативные процессы эндометрия, гиперплазия эндометрия, постменопауза, гормональная терапия, ERα, ERß, mER, PRA, PRB, mPR, PGRmC1

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Endometrial cancer occupies a prominent place in the epidemiological structure of malignant tumours [1]. The morbidity peaks at 65–69 years, constituting 98.1 cases per 100,000 women of this age group. Endometrial adenocarcinoma is the most prevalent type of uterine cancer. This hormone-dependent tumor often develops against the clinical background of endometrial proliferative process (EPP) [2]. The hormone therapy options show limited efficacy for endometrial tumours and are indicated exclusively in young patients with early stages of low-grade uterine cancer. In cases of atypical endometrial hyperplasia (AEH), surgical treatment is usually recommended first-line due to the high risks (40–60%) of concomitant endometrial adenocarcinoma [3]. For non-atypical endometrial hyperplasia (EH), hormone therapy may be 100% efficacious; however, to achieve this level, the progestogens must act directly and sustainably on the endometrium (e.g. by means of levonorgestrel-containing intrauterine system) [4, 5]. With the use of oral forms, the efficacy constitutes 50–69% [6]. The higher rates of response achieved with intrauterine delivery systems can be attributed to their low systemic influence.

Personalized prediction of response to hormone therapy in EPP is highly relevant. The candidate prognostic factors include expression of pro- and antiapoptotic proteins and overall endometrial receptivity, as well as differential expression of hormone receptors in endometrial glands and stroma [7–11]. However, no convincing results at the tissue level have been obtained so far. Even with the high sensitivity of tissue to hormonal influence, systemic effects of the treatment were often disregarded. In particular, data on the immunity-related side effects of hormone therapy for EPP are missing, despite its alleged principal significance. Despite the lack of significant correlation between the type of endometrial pathology and composition of immunocompetent cells, the corresponding functional relationships have not been assessed [12].

According to recent evidence, hormone substances (both endogenous and pharmaceutical) affect immunocompetent cell functionalities as they bind corresponding receptors abundantly expressed by these cells [13], and the outcomes may be complex, as different cell types and subpopulations of the immune system express specific hormone receptor signatures [13]. It should be noted, however, that these preliminary data were collected in healthy donors without proper accounting for the age group.

The aim of this study was to assess the feasibility and advisability of hormone therapy for EPP in postmenopausal patients on personalised basis, by studying differential expression of estradiol and progesterone receptor genes in endometrial tissue and peripheral blood mononuclear cells (PBMC) collected from the patients. In addition, we applied in vitro tests of the hormone drug effects on PBMC receptor profiles as a sensitivity assay.

METHODS

The study was carried out in 2012–2021 at the City Clinical Hospital № 31, Moscow, the clinical base for the Obstetrics and Gynecology Chair of the Pirogov Medical University. Part of the material was accessed in the Oncogynecology Department of the Oncology Dispensary № 1, Moscow. The study enrolled 92 hospitalized patients with EPP, postmenopausal, assigned to either main or comparison group based on histological diagnosis. The main group included 55 pts with different forms of high-rate EPP: 7 pts with non-atypical endometrial hyperplasia (EH), 8 pts with atypical endometrial hyperplasia (AEH) and 40 pts with endometrial adenocarcinoma (31 pts with moderately differentiated adenocarcinoma (G1) and 9 pts with highly differentiated adenocarcinoma (G2)). The comparison group included 37 pts with histologically verified glandular-fibrous endometrial polyps qualified as a low-rate form of EPP. The study of hormone receptor gene expression in PBMC involved a control group of 10 healthy women in postmenopause without gynecological pathology. This group consisted of staff members at the Pirogov Medical University and City Clinical Hospital № 31 without clinical manifestations of EPP. The absence of endometrial pathology and other gynecological diseases was confirmed by pelvic ultrasound scans. The control group donated blood samples.

Inclusion criteria for main and comparison groups were as follows: the presence of pathomorphologically verified endometrial pathology; post-menopause. The histological specimens were classified in accordance with the 2014 World Health Organization (WHO) classification criteria as non-atypical endometrial hyperplasia, atypical endometrial hyperplasia, endometrial adenocarcinoma (either moderately or highly differentiated) or endometrial polyp (glandular-fibrous variant).

Exclusion from the study (main, comparison and control groups) was based on the following unified criteria: taking hormonal medications (estrogens, gestagens, gonadotropin-releasing hormone agonists, hormone replacement therapy and/or tamoxifen) within 6 months prior to the study, gynaecological diseases (uterine fibroids of size exceeding a 6–7 weeks pregnant uterus, ovarian tumors) and inflammatory processes of any localisation at the time of sampling.

The age of participants enrolled in the study varied within the total range of 53–80 years constituting 64.2 ± 6.27 years on average. The time since menopause varied and depended on the age of participants.

The reasons for hospitalisation included endometrial pathology confirmed by pelvic ultrasound examination in 60 pts (65.22%) and genital tract bleeding in 32 pts (34.78%).

The gynaecological comorbidities were dominated by small uterine fibroids (5- to 6-week pregnancy and at the stage of regression) in 63.04%. In addition, 28.26% of the patients had a history of endometrial pathology (polyps, non-atypical endometrial hyperplasia) at various times in their lives.

The non-gynecological comorbidities were dominated by chronic hypertension, ischemic heart disease and variable degree of obesity (diagnosed in 80.43%, 72.82% and 90.2% of the patients, respectively).

All patients underwent standard examination including routine ultrasound scans with a Logiq E9 apparatus (GE; USA) equipped with a 4–10 MHz intracavitary microconvex transducer. All patients presented with increased endometrial thickness exceeding 4 mm, accompanied by echostructure heterogeneity and hyperechoic foci.

Hysteroscopy with dilation and curettage was performed in 55 (59.78%) pts, 16 of which underwent resection or ablation of the endometrium. Total hysterectomy was performed in 37 (40.22%) pts by laparotomy in 30 pts, laparoscopy in 6 pts and vaginal access in 1 pt.

Blood samples were collected from the patients before the surgery. PBMC were isolated from peripheral blood as described by Bouman et al. [14]. The endometrial biopsies were dissected midway into identical halves: one for gene expression assay and one for histological examination. The tissues were placed into labeled Thermo Scientific™ Nunc™ 1.5 mL cryogenic vials and frozen in liquid nitrogen to preserve mRNA, subsequently isolated with RIBO-prep kits (InterLabService; Russia) and handled in accordance with manufacturer's protocols.

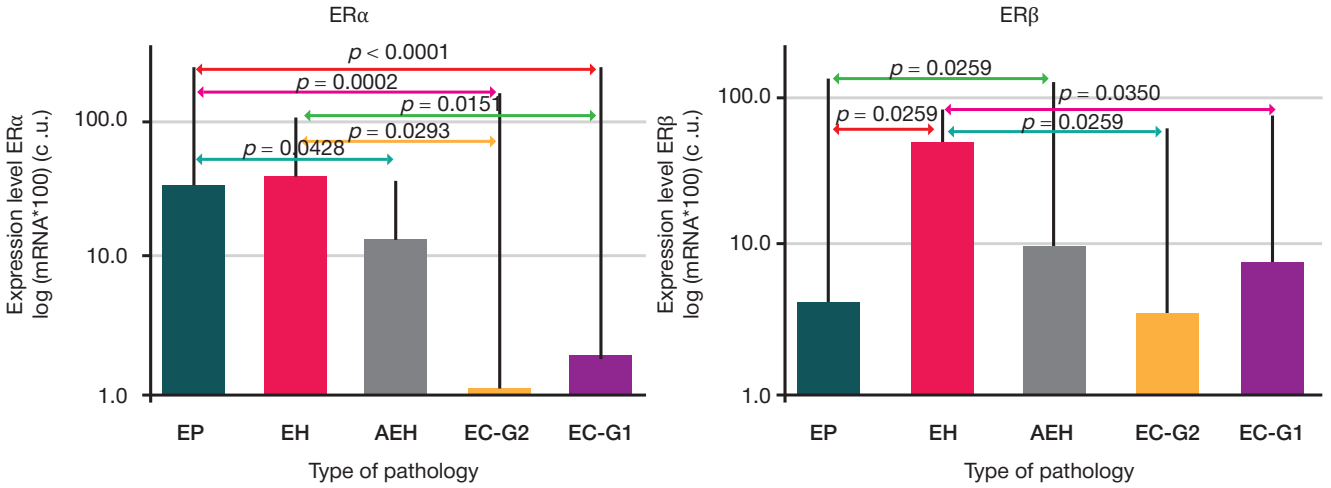

Fig. 1. Expression of estradiol nuclear receptor genes in endometrial tissue of postmenopausal patients with endometrial proliferative process. Vertical axis: lg mRNA level (1/2–ΔCt) × 10^2, reference gene GAPDH; horizontal axis: EP — endometrial polyp (glandular-fibrous), EH — non-atypical endometrial hyperplasia, AEH — atypical endometrial hyperplasia, EC-G1 — highly differentiated (G1) endometrial carcinoma, EC-G2 — moderately differentiated (G2) endometrial carcinoma

Expression levels of estradiol and progesterone receptor-encoding genes (respectively ERα, ERβ and mER, and PRA, PRB, mPR and PGRmC1) in endometrial biopsies and PBMC were assessed by real time polymerase chain reaction method (RT–PCR). mRNA isolation, reverse transcription and DNA quantitation were carried out by standard protocols; gene expression levels were assessed by PCR using iCycler iQ™ Real Time PCR system (BioRad; Germany) against GAPDH as a reference transcript. The relative abundancies of specific transcripts were calculated by ΔCt method using (1/2)^ΔCt and 2^(–ΔΔCt) equations, where ΔCt = Ct(mER) – Ct(GAPDH) and ΔΔCt = ΔCt (pathology) – ΔCt (control).

In addition, we conducted in vitro experiments to assess the effects of gestagen exposure on estradiol and progesterone receptor gene expression in PBMC isolated from blood samples of the patients with endometrial polyps and matching healthy individuals. The cells were exposed to progesterone or mifepristone added to 10^{-8} M final concentrations in the incubation medium to be compared with no-hormone-added control incubations. Following the exposure, the cells were subject to estradiol and progesterone receptor gene expression measurements as described in the previous paragraph.

PBMC viability *in vitro* was measured by MTT test [15] following the exposure.

Patient database for the study was constructed on the basis of individual clinical histories, with the use of standard program packages Microsoft Excel 7.0 (Microsoft; USA) for Windows 2007 (Microsoft; USA) and Numbers version 4.3 (5046) (Apple; USA) for MacBook Pro 2013 (Apple; USA). The analysis involved comparative evaluation of qualitative and quantitative indicators for the groups. Statistical processing and visualisation of the data was carried out in GraphPad Prism version 5.0 (GraphPad Software; USA) and STATISTICA 8 (StatSoftInc; USA) program packages. The comparisons were made using Fisher and Mann–Whitney tests; differences qualified as non-random with at least 95% probability ($p < 0.05$) were considered statistically significant.

RESULTS

The results indicate that expression of estradiol and progesterone receptor genes in endometrial tissue depends on the types of endometrial pathology. Consistently with previous findings [16], high expression levels of estradiol receptors ERα and ERβ were observed in EH and low in adenocarcinoma (Fig. 1).

For progesterone nuclear receptor genes [16], we revealed high expression levels in EH and AEH (Fig. 2). The PRA and PRB expression levels, although similar between the groups, differed in a more specialised comparison between patients with adenocarcinoma and AEH. Consistently with other studies, we observed a sharp decrease in expression for PRA and a less pronounced effect for PRB in adenocarcinoma compared with AEH.

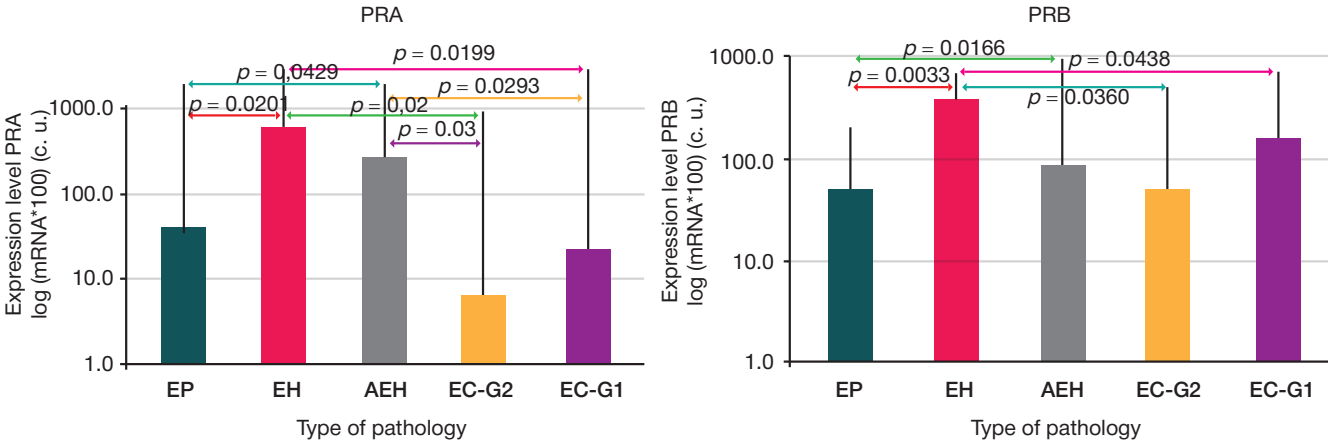

Fig. 2. Expression of progesterone nuclear receptor genes in endometrial tissue of postmenopausal patients with endometrial proliferative process. Vertical axis: lg mRNA level (1/2–ΔCt) × 10^2, reference gene GAPDH; horizontal axis: EP — endometrial polyp (glandular-fibrous), EH — non-atypical endometrial hyperplasia, AEH — atypical endometrial hyperplasia, EC-G1 — highly differentiated (G1) endometrial carcinoma, EC-G2 — moderately differentiated (G2) endometrial carcinoma

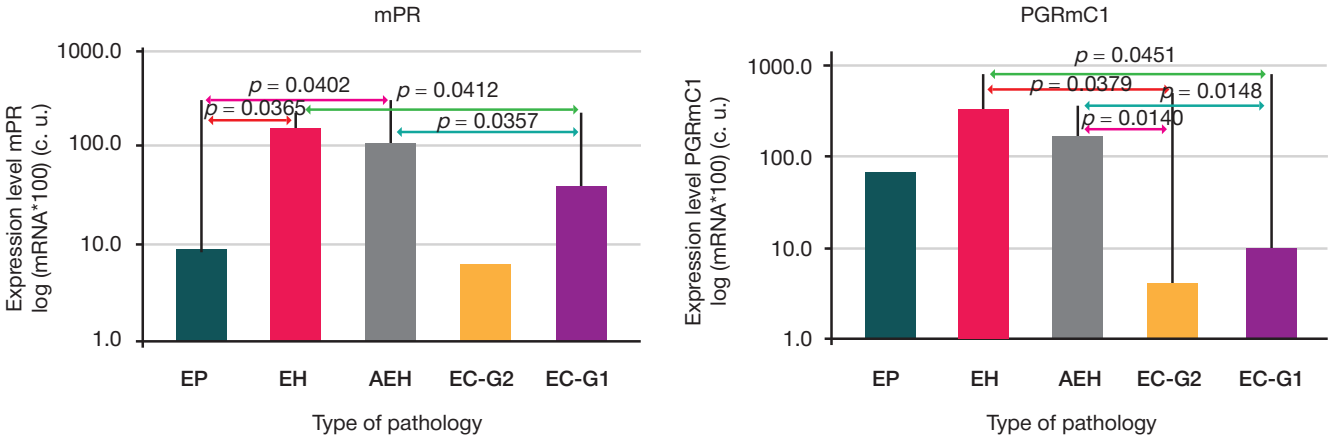

Fig. 3. Expression of progesterone membrane receptor genes in endometrial tissue of postmenopausal patients with endometrial proliferative process. Vertical axis: lg mRNA level (1/2–ΔCt) × 10^2, reference gene GAPDH; horizontal axis: EP — endometrial polyp (glandular-fibrous), EH — non-atypical endometrial hyperplasia, AEH — atypical endometrial hyperplasia, EC-G1 — highly differentiated (G1) endometrial carcinoma, EC-G2 — moderately differentiated (G2) endometrial carcinoma

We also observed elevated expression of mPR in EH and AEH (Fig. 3), whereas PGRmC1 expression levels were specifically reduced in adenocarcinoma (Fig. 3).

Consistently with previous reports [17], PBMC isolated from patients with AEH expressed higher levels of mER, ERα and PRA compared with the cells derived from healthy women (Fig. 4). In addition, PBMC isolated from patients with endometrial adenocarcinoma expressed significantly lower levels of ERβ compared with the control group.

Comparison of estradiol and progesterone receptor gene expression levels between endometrial tissue and PBMC revealed matching profiles for ERα and PRA (Figs. 1, 2, 4).

In vitro exposure of PBMC derived from healthy women to either mifepristone or progesterone stimulated the expression of both estradiol and progesterone receptor genes (Figs. 5, 6).

A similar exposure of PBMC derived from patients with endometrial polyps to progesterone resulted in elevated expression of mER, mPR and PGRmC1, whereas exposure of these cells to mifepristone resulted in decreased expression of ERα, ERβ, mPR, PGRmC1, PRA and PRB.

In vitro study of mifepristone effects on PBMC viability using MTT test revealed a decrease in the number of viable proliferating cytokine-producing immunocompetent cells ($p < 0.05$).

DISCUSSION

The proper, patient-oriented selection of hormone therapy in postmenopausal patients with EPP requires accounting for the steroid receptor status of endometrial tissue. With low

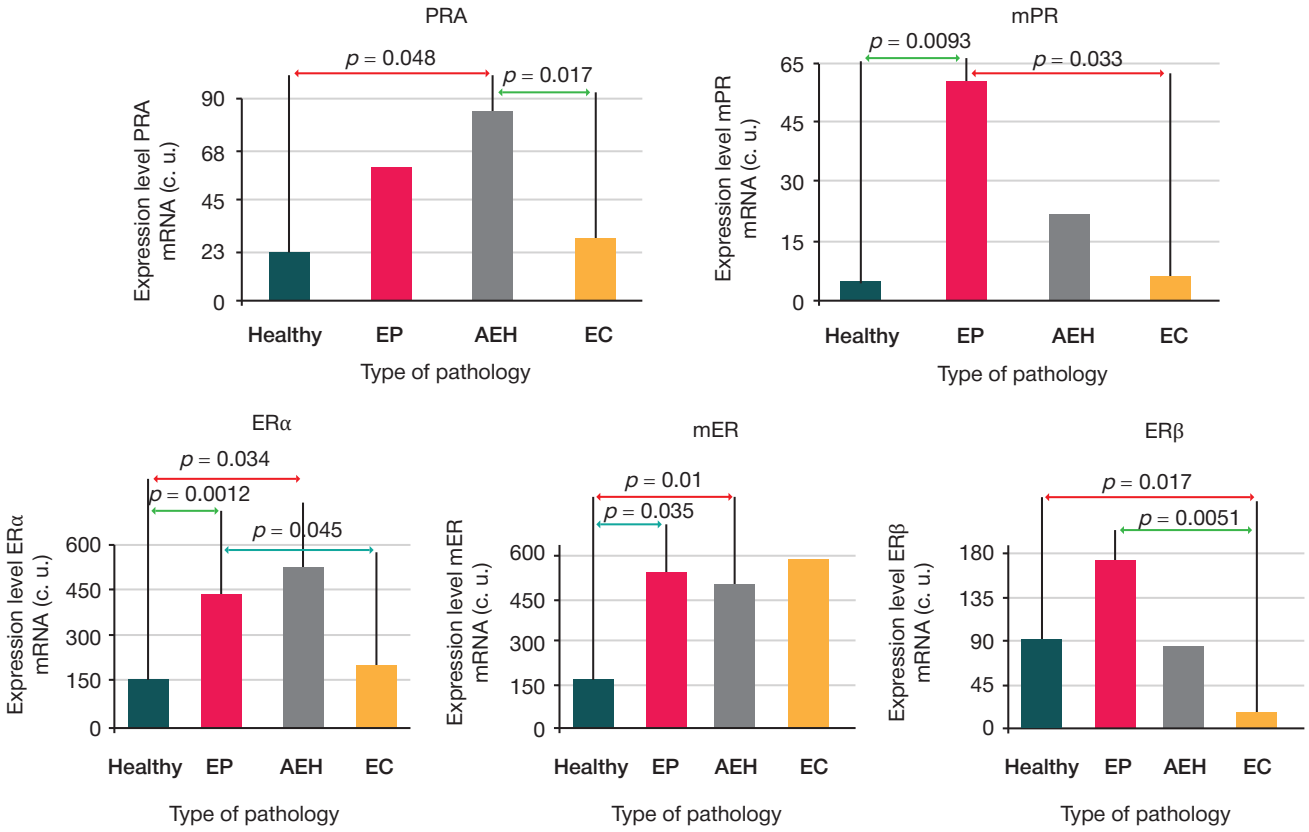

Fig. 4. Expression of estradiol and progesterone receptor genes in peripheral blood mononuclear cells of postmenopausal patients with endometrial proliferative process. Vertical axis: lg mRNA level (1/2–ΔCt) × 10^4, reference gene GAPDH; horizontal axis: Healthy — matching healthy donors; EP — endometrial polyp (glandular-fibrous), AEH — atypical endometrial hyperplasia, EC— endometrial carcinoma; *p* — level of significance

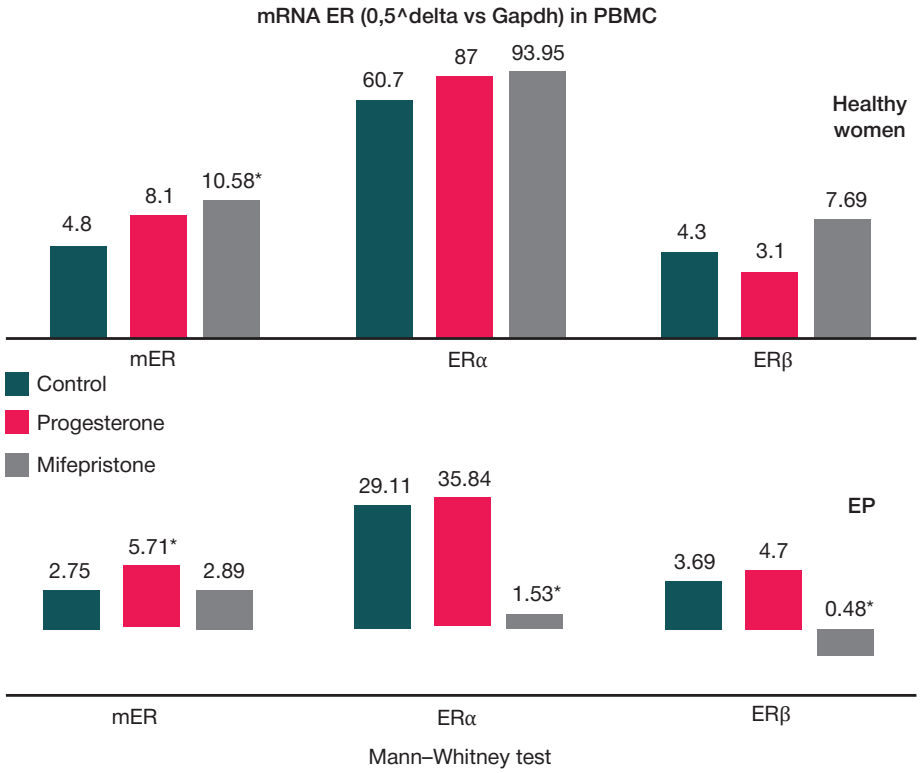

Fig. 5. Expression of estradiol receptor genes in peripheral blood mononuclear cells after *in vitro* exposure to progesterone and mifepristone. Vertical axis: mRNA level (1/2–ΔCt) × 100, reference gene GAPDH; horizontal axis: receptor type; EP — endometrial polyp (glandular-fibrous); * — $p \leq 0.05$

receptor availability, the local response to hormone exposure may not reach therapeutic values [4, 6, 7]. However, some postmenopausal patients with EPP show resistance to hormone therapy and develop relapses despite the availability of hormone receptors in the endometrial tissue. Such clinical situations are mechanistically unclear and require further investigation.

ERα and ERβ are main receptors that mediate proliferative action of estrogens on the endometrial tissue [18], with the major role played by ERα. The high expression of ERα and ERβ observed by us in EH and AEH (Fig. 1) may indicate potential efficacy of aromatase inhibitors (estrogen synthesis blockers) in postmenopausal patients with this condition.

The elevated expression levels of ERβ in EH and AEH (Fig. 1) indicate its involvement in EPP pathogenesis. ERβ is known to mediate both proliferative and anti-proliferative estrogen signaling, which makes it a plausible drug target and justifies the screenings for its selective ligands. A correlation of higher ERβ expression levels with more aggressive forms of endometrial adenocarcinoma, demonstrated by comparative analysis

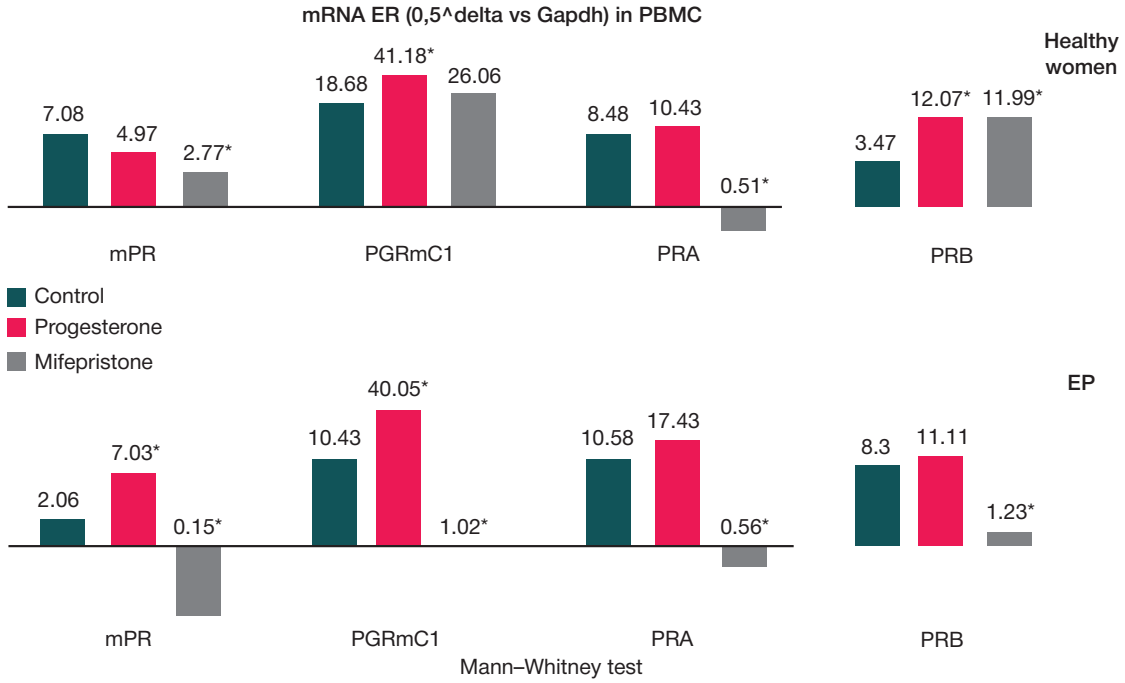

Fig. 6. Expression of progesterone receptor genes in peripheral blood mononuclear cells after *in vitro* exposure to progesterone and mifepristone. Vertical axis: mRNA level (1/2–ΔCt) × 100, reference gene GAPDH; horizontal axis: receptor type; EP — endometrial polyp (glandular-fibrous); * — $p \leq 0.05$

of ERα and ERβ expression in endometrial adenocarcinoma tissues [18], corroborates our hypothesis on a prominent role of ERβ in EPP pathogenesis. Other authors report high expression of estradiol nuclear receptors in EH tissues revealed by immunohistochemistry [19] albeit in mixed-age cohorts.

High expression levels of progesterone nuclear receptors in EH and AEH observed by us in this study (Fig. 2) may represent a compensatory counter-proliferative mechanism, possibly indicating a shortage of endogenous gestagens and justifying the prescription of synthetic gestagens as a pathogenetic treatment in this form of endometrial pathology. At the same time, in adenocarcinoma, PRB shows no sharp decrease in expression (by contrast with PRA), and is expressed at a level comparable with AEH, which may reflect unequal contributions of PRA and PRB to the pathogenesis consistently with other reports on this subject [20]. For instance, in the absence of PRA, PRB can support proliferation [21]. In this regard, given the predominance of PRB expression in endometrial tissue under conditions of EH, it would be reasonable to expect the lack of inhibitory effect from progesterone. In such cases, the gestagen-based regimens are likely to fail and even promote disease progression. These considerations indicate the need for personalised profiling of steroid receptors in endometrial biopsies or curettage material prior to synthetic gestagen prescription. At high PRA expression levels the gestagen therapy administration can be justified, whereas at high PRB expression levels its benefits should be doubted.

The membrane progesterone receptor mPR can potentiate the PRB-mediated signal transmission and significantly contribute to the progression of endometrial adenocarcinoma [22]. mPR and PRB jointly mediate the gestagen-induced proliferative signaling in target cells. Accordingly, the observed high expression level of mPR in EH and AEH (Fig. 3) may represent an adverse factor along with high expression of PRB.

Decreased expression of PRA, mPR and PGRmC1 in endometrial adenocarcinoma tissue indicates its compromised receptivity to progesterone and explains the low efficacy of hormone therapy for these patients, consistently with the common opinion that prescriptions of hormone therapy for endometrial adenocarcinoma should involve hormone receptor gene expression profiling administered on personalized basis [11].

Proliferative processes are known to be controlled by the immune system. Under compromised immune surveillance, these processes may become uncontrollable and lead to malignant transformation of the tissue. The receptor-mediated influence of sex steroids on immunocompetent cells is a proven fact [13]. However, to the best of our knowledge, no explicit data on the expression of estradiol and progesterone receptors by PBMC in EPP are available as yet.

In this study we observed impaired sensitivity of PBMC to steroids in EPP, indicative of their altered functionalities, given the reportedly unaltered leukocyte formula in EPP [12]. The unique receptor signatures expressed by different types of mononuclear cells [13] help explain the differential changes in their functionalities under the action of hormones.

ERα is the dominant type of estradiol receptors in monocytes/macrophages and T-helpers. Estradiol suppresses the monocyte-macrophageal responses while stimulating T-helper immunity [23, 24]. The high expression of ERα by PBMC in AEH observed by us in this study (Fig. 4) indicates increased sensitivity of the cells to the inhibitory action of estradiol on the monocyte-macrophageal compartment along with the opposite, stimulating effect on T-helper immunity. On the other hand, such stimulation of T-cell responses is known to activate macrophages and promote the release of pro-inflammatory cytokines by these cells second-hand [23, 24], which explains the persistence of chronic inflammatory processes as one of pathogenetic components in malignant tumorigenesis.

T-killers, known to be heavily engaged at early stages of the anti-tumor response, are almost entirely devoid of nuclear receptors to estradiol; meanwhile, estrogens effectively suppress the activity of T-killers [24]. These findings implicate the membrane receptor mER as a cornerstone of estrogen signaling in T-killers. Accordingly, the increase in mononuclear cell sensitivity observed by us for AEH (Fig. 4) may favor the clonal preservation of atypical cells in endometrial tissue.

PRA has been shown to mediate the inhibition of cytokine release by progesterone [25]; among PBMC, this receptor is expressed by natural killers only. The high expression level of PRA observed by us in natural killer cells (Fig. 4) apparently potentiates the AEH progression.

Thus, in AEH, the monocyte, macrophage, T-cell and natural killer cell functionalities are inhibited. The explanation runs as follows: hyperexpression of receptors enhances the sensitivity of mononuclear cells to hormones (endogenous and pharmaceutical), which ultimately leads to the inhibition of immunocompetent cell functionalities. The uncontrollable nature of this process may support AEH malignisation, hence the low efficacy (and occasionally the overall adverse effect) of hormone therapy in postmenopausal patients with AEH.

In contrast to AEH, expression levels of ERα, PRA, mPR by PBMC in adenocarcinoma are similar to those of healthy women (Fig. 4), which is rather unexpected given the progressive deterioration of immunity characteristic of cancers. The dominance of ERα and PRA in PBMC has been already mentioned. According to the published evidence, mPR is a major steroid receptor in T-cells and monocytes/macrophages [26]. In combination with our data, this means that corresponding transcriptomic signatures of monocytes, macrophages, T cells and normal killer cells in postmenopausal patients with endometrial adenocarcinoma stay unaltered. This feature may be considered as a sign of escape of the malignant tumor from the immune surveillance.

Unexpected disease-related expression dynamics in PBMC was revealed for ERβ (Fig. 4). This receptor is the principal member of estrogen signaling pathway in B-cells [27]. Our data reveal a sharp decrease in its expression for endometrial adenocarcinoma compared to the control group (Fig. 4). In our opinion, this indicates hardly a loss of tumor sensitivity to external signals (since in such case the receptor expression levels would be closer to normal), but rather a second-hand suppression of B-cell immunity by the tumor.

Comparative analysis of hormone receptor gene expression in endometrial tissue and PBMC revealed matching ERα and PRA profiles. For endometrial adenocarcinoma, the trend may indicate masking of the tumor from direct effects of hormones as well as the immune system. Accordingly, prescription of hormone therapy to postmenopausal patients with endometrial adenocarcinoma is unjustified.

The high expression of PRA by endometrial tissue and mononuclear cells in AEH (Figs. 2, 4) indicates their increased sensitivity to gestagens; in PBMC, this feature is conducive to the atypical clone preservation.

Thus, pharmacological strategies for EPP in postmenopausal patients should account for the immunity-related side effects involving PBMC. The hormone drugs should be prescribed with caution due to their possible negative effect on immunocompetent cells.

To determine a synthetic drug with the mildest effect on the immune system, we comparatively analysed the binding

activity of progesterone receptors in PBMC with gestagens (P4 = 100%) and observed a 2-times weaker PBMC binding affinity for the so-called strong gestagens (medroxyprogesterone acetate and norethisterone, commonly prescribed in EPP) compared to progesterone ($p < 0.05$). As these drugs have been shown to effectively side-target immunocompetent cells and thus reduce their anti-proliferative activity, our experimental study engaged the anti-gestagenic drug mifepristone chosen as a model candidate medication to gain control over these processes.

Mifepristone, which actively and effectively binds progesterone receptors in mononuclear cells, is widely featured in clinical studies of treatments for hormone-dependent pathologies [28]. We used the 'canonical' mifepriston rather than more recent formulations because of its antitumor activity and lack of hepatotoxicity [29], which is particularly important in postmenopausal patients.

Progesterone exposure of PBMC derived from healthy women and patients with endometrial polyps promoted an increase in estradiol and progesterone receptor gene expression in both sample types (Figs. 5, 6), indicating increase sensitivity to the adverse impact of sex steroid hormones on PBMC functionalities. The differential influence of varying progesterone concentrations on cytokine synthesis has been demonstrated by other authors [30]; our findings (we used progesterone in nanomolar concentrations) do not contradict the published evidence.

Mifepristone exposure of PBMC revealed a beneficial effect of this drug on cells derived from patients with endometrial polyps, in the form of decreased expression of ERα, ERβ, mPR, PGRmC1, PRA and PRB receptors in PBMC (Figs. 5, 6), possibly counteracting the negative effect of sex steroids on immunocompetent cells. This observation justifies the prescription of this drug to patients with endometrial pathology, aimed at mitigation of PBMC sensitivity to suppressive action of sex hormones and thus promoting normalisation of the immune status. The data requires further validation on clinical cohorts.

The suppressive effect of mifepristone on the mononuclear cell viability observed by us in this study requires further investigation in order to screen for related substances most sparing towards the immunocompetent cells, but at the same time reducing the increased expression of estradiol and progesterone receptors by PBMC.

CONCLUSIONS

Selection of pathogenetic hormone therapy for endometrial proliferative process in postmenopausal patients requires accurate assessment of receptor status in the endometrial tissue and also in peripheral blood mononuclear cells. Hormone receptor gene expression data are highly relevant for predicting the response of endometrial tissue to hormone therapy in combination with its effect on immunocompetent cells. According to the results, high levels of PRA mRNA in endometrial hyperplasia sample can be used as a predictor of efficacy for anti-relapse gestagens therapy. Most notably, at high expression levels of PRB, its can be dangerous — leading to unpredictable tissue response and progressive proliferation. The cause of non-efficacy of the standard gestagen therapy for endometrial proliferative process is partially due to its underestimated inhibitory influence on the immune system. The effects of hormone drugs on functional profiles of PBMC and neutralisation of the adverse side effects of sex steroid hormones on immunocompetent cells without reducing their viability are relevant subjects of further investigation.

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