Disorders in Cytokine Gene Expression in Endometrial Hyperplasia and Effect of Hormone Therapy

G. T. Sukhikh, A. V. Zhdanov, M. P. Davydova*, T. V. Slukina, G. E. Chernukha, T. E. Samoilova, and V. P. Smetnik

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 2, pp. 204-207, February, 2005
Original article submitted June 2, 2003

We studied local expression of insulin-like growth factor 1, insulin-like growth factor receptor, epithelial growth factor, transforming growth factor β_2, PCNA, TNF-α, type I TNF receptor, Fas, FasL, IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, and IL-12 genes in intact and hyperplastic endometrium. Endometrial hyperplasia was associated with reduced production of TNF-α ($p<0.05$), PCNA ($p<0.05$), and epithelial growth factor mRNA and enhanced production of Fas mRNA ($p<0.01$). The expression of TNF-R1, IL-1β, and IL-12 genes decreased only in glandular cystic hyperplasia ($p<0.05$ for all genes), expression of insulin-like growth factor 1 gene decreased only in adenomatous hyperplasia ($p<0.05$). Dufaston therapy of glandular cystic hyperplasia and zoladex therapy of adenomatous hyperplasia normalized expression of Fas receptor, PCNA, and insulin-like growth factor 1 genes, while the expression of IFN-γ and IL-6 genes, which was normal in hyperplasia, decreased ($p<0.05$). Zoladex therapy decreased the production of transforming growth factor β_2 ($p<0.05$) and IL-1β ($p<0.01$) mRNA, dufaston therapy decreased production of TNF-α ($p<0.05$) and IL-4 mRNA ($p<0.05$). Hence, both apoptosis and proliferative activity were suppressed in endometrial hyperplasia, and hormone therapy created prerequisites for transition of the endometrium into the normal proliferation stage.

Key Words: *mRNA; cytokines; endometrial hyperplasia*

Hyperplastic processes occupy one of the first places in the structure of gynecological diseases. Endometrial hyperplasia (EHP) develops in 5-15% women. This condition in the overwhelming majority of cases is characterized by menstrual cycle disorders of the oligomenorrhea type alternating with menometrorrhagias against the background of relative hyperestrogenia also caused by chronic anovulation. EHP risk factors are polycystic ovary syndrome, obesity, and type II diabetes mellitus [5]. However, in 30-40% cases EHP develops in the absence of hormonal and metabolic disorders, which implies multifactorial genesis of hyperplastic processes.

Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences; *Faculty of Fundamental Medicine, M. V. Lomonosov Moscow State University, Moscow

Changes in the expression of factors regulating endometrial status during the menstrual cycle during EHP still remain unclear. There are good grounds to hypothesize that these changes involve mainly the growth and apoptosis factors. For example, the expression of PCNA and Ki-67 proliferation activator genes in endometrial hyperplasia decreases in comparison with the proliferation stage, especially in atypical hyperplasia (AH) [6,10]. Some authors reported increased production of epithelial growth factor (EGF) in EHP [12]; the data on insulin-like growth factor 1 (IGF-1) (apoptosis inhibitor and proliferation factor) are contradictory [7,12]. On the whole, the role of cytokines and apoptosis factors in the development of endometrial hyperplasia is virtually not studied.

We carried out a semiquantitative study of the local expression of genes of 16 factors involved in the

0007-4888/05/1392-0235

morphofunctional regulation in normal and hyperplastic endometrium samples from patients with glandular cystic hyperplasia (GCH) and AH before and after dufaston and zoladex therapy.

MATERIALS AND METHODS

Specimens of endometrium from women with GCH (n=17) and AH (n=12) were examined. All patients presented with chronic menstrual cycle disorders and high incidence of relapses (1-9, 3.03±1.84 on average). Specimens of the endometrium from women of reproductive age without endometrial diseases and infections, collected at the stage of medium and late proliferation (n=5; control) and early secretion (n=5; reference group) served as the control. Patients with GCH received dufaston in a daily dose of 20 mg on cycle days 5-25 or 12-25, patients with AH were treated with zoladex (3-6 subcutaneous injections, 3.6 mg, once in 28 days).

The expression of the following genes was studied: IGF-1 proliferation activate, insulin-like growth factor receptor (IGF-R), EGF, transforming growth factor (TGF-β_2), PCNA; TNF-α, TNF-R1, Fas, FasL, IFN-γ (proliferation suppression and apoptosis activation factors); IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12 (menstrual cycle and inflammatory process regulators). Primer sequences were reported previously [3,4], except 5'-GGTCTTGGGCATGTCGGTGTG and 5'-TGTCCTCCTCGCATCTCTTCTACCTG (IGF-1, 317 n. p.); 5'-TGCCCG-TCGCTGTCCTGT TG and 5'-GCTTGCGGCCTCGTTCACTGT (IGF-R, 302 n. p.); 5'-TGCTGCTCACTCTTATCATTCTGT TGC and 5'-AATGATTCCTTC-CTGTTGATTTGAC CA (EGF, 419 n. p.); 5'-GCCTGTAGCGGCGTTGT TGC and 5'-CGCGTTATCTTCGGCCCTTAGTG (PCNA, 320 n. p.).

The study was carried out for all samples simultaneously. RNA isolation, DNase treatment, reverse transcription and PCR were carried out as described previously [1,4] using TRI REAGENT™ (Sigma), RQ1 DNAse (Promega), MMLV reverse transcriptase (Promega), hexarandome- and oligo-dT primers. PCR was carried out in PCR Express thermocyclers (Hybaid). PCR results were analyzed by electrophoresis and documented using Gel Doc 1000 system (Bio-Rad). Production of mRNA for each factor during the secretion stage was taken as one unit. Quantitative analysis and statistical processing of the data were described previously [4].

RESULTS

The expression of PCNA and IGF-1 proliferation factors and Fas apoptosis inductor in normal endometrium samples was significantly higher during the proliferation stage than during the secretion stage (p<0.05). The expression of other 12 genes was similar in the control and reference groups.

Local expression of some cytokine genes was changed in patients with hyperplasia in comparison with normal endometrium during the proliferation stage. The direction of changes in GCH and AH in general coincided, though with some exceptions: the expression of IGF-1 gene was lower in AH (p<0.01 *vs.* GCH and p<0.05 *vs.* control), while the production of IL-12 mRNA in GCH was significantly lowered (p<0.01 *vs.* AH and p<0.05 *vs.* control), as well as of IL-1β mRNA (p<0.05 *vs.* AH and control).

The expression of Fas receptor gene markedly increased in hyperplastic endometrium (30.3 times in GCH, p<0.05; 15.8 times in AH, p<0.05), while the expression of Fas ligand virtually did not differ from normal. The expression of TNF-α significantly decreased both in GCH and AH (5.5 and 24 times, respectively, p<0.05). The level of type I TNF-α receptor mRNA was significantly reduced only in GCH (p<0.05), while in AH this parameter only tended to decrease.

The expression of growth factors was decreased in hyperplasia: PCNA: 8.7 times in GCH and 15.6 times in AH (p<0.05 and p<0.001, respectively); IGF-1: 1.6 times in GCH (negligibly) and 3-fold in AH (p<0.05). The expression of EGF gene was markedly reduced in both forms of hyperplasia, but we failed to detect the differences by statistical methods because the groups were very small. EGF gene was expressed in 50% endometrial specimens at the secretion stage, in 75% specimens at the proliferation stage, in 40% of GCH, and in 50% of AH samples. Changes in the expression of IGF-1 receptor were negligible.

The production of IL-1β mRNA decreased 32 times in GCH (p<0.01), while in AH this parameter only tended to decrease. The expression of IL-12 gene in GCH also markedly decreased (7.4 times, p<0.01). The expression of IL-4, IL-6, IL-8, IL-10, TGF-β_2, and IFN-γ genes in hyperplasia of different types did not differ significantly from the levels in normally proliferating endometrium.

In general the production of many factors activating cell proliferation and preventing apoptosis is appreciably reduced in EHP. This is true, first and foremost, for PCNA, an obligatory element of the replicative complex [11]. Our findings are in line with published data on decreased mitotic activity and PCNA production in hyperplasia [6]. By contrast, the detected decreased expression of IGF-1 and EGF genes was never noted before [9,12]. On the other hand, an appreciable increase in the Fas mRNA production seems to indicate high readiness of cells to receive a signal triggering programmed death. Since IGF-1 is an apo-

ptosis blocker, the decreased expression of the corresponding gene is an indirect prerequisite for activation of apoptosis in hyperplastic endometrium. However, it is known that apoptosis in EHP increases just negligibly in comparison with the proliferation stage [8]. Its negative regulation in EHP can be seen from low activity of Ca^{2+}/Mg^{2+}-dependent endonucleases [2] participating in DNA degradation. The causes of apoptosis inhibition can be, among other things, decreased expression of TNF-α or insufficient production of FasL and TNF-R1, revealed in our study.

Dufaston therapy in GCH, similarly as zoladex therapy in AH, led to changes in the expression of Fas receptor, PCNA, and IGF-1 towards their normalization. The decrease of Fas expression was significant in both groups (37.6 times in GCH, $p<0.01$, and 24.9 times in AH, $p<0.05$). The increase of IGF-1 gene expression was also significant after therapy with zoladex and dufaston: 6- ($p<0.01$) and 3.7-fold ($p<0.05$), respectively. On the other hand, the increase of PCNA expression observed in both groups was significant only after zoladex therapy of AH patients (4.8 times, $p<0.05$). Expression of IFN-γ and IL-6 decreased significantly in the GCH group (1.9 and 14.3 times, respectively, $p<0.05$) and in AH group (1.6 and 91 times, respectively, $p<0.05$), though before therapy the production of mRNA for these cytokines did not differ from normal. The expression of TGF-β_2 and IL-1β decreased significantly ($p<0.05$ and $p<0.01$, respectively) after zoladex therapy of AH patients; the initial production of TGF-β_2 mRNA was slightly higher and of IL-1β mRNA lower than in the control. Dufaston therapy was associated with further suppression of TNF-α ($p<0.05$) and IL-4 ($p<0.05$) genes expression.

Hence, expression of many of the studied factors remained low after the treatment in comparison with the level characteristic of the proliferation and secretion stages in intact endometrium (TNF-α, TNF-R1, IFN-γ, IL-1β, IL-4, IL-6, EGF). In some cases suppression of gene expression was even deeper than in EHP. The production of FasL, IL-8, IL-10, IL-12, IGF-R, and EGF mRNA was resistant to therapy with drugs modulating the hormonal status. It seems that the expression of some these genes cannot be regulated by sex steroid hormones, as its level virtually did not change in hyperplasia (FasL, IL-8, IL-10, IGF-R). We conclude that in general the therapy led to a decrease in the expression of factors capable of inducing apoptosis or suppressing cell growth (TNF-α, IFN-γ) and an increase in the levels of some factors maintaining proliferation and inhibiting apoptosis (PCNA, IGF-1). These changes can be regarded as an evidence of transition of the endometrium into the proliferation phase. Morphological analysis indicated that 70% of endometrial specimens after dufaston therapy and 44.4% specimens after zoladex therapy corresponded to the proliferative stage.

These data indicate that many-fold increase of Fas production in endometrial hyperplasia, typically not characterized by high level of apoptosis, is not a signal triggering programmed death. On the other hand, EHP is associated with suppression of proliferative activity, which is seen from decreased expression of cell proliferation factor genes.

Hence, it seems that endometrial hyperplasia is a condition when cell proliferation and apoptosis (both processes whose consecutive activation maintains normal functioning of the endometrium) are inhibited. Hormone therapy has a certain positive effect on the expression of some genes encoding cell proliferation and death factors, due to which the recovery of the menstrual cycle becomes potentially possible. On the other hand, revision of current views on the origin of hyperplasia and understanding of EHP as a phenomenon of simultaneous inhibition of apoptosis and proliferation can stimulate the improvement of traditional and development of principally novel approaches to therapy.

REFERENCES

1. A. V. Zhdanov, F. G. Gadiati, D. A. Varlamov, *et al.*, *Byull. Eksp. Biol. Med.*, **131**, No. 3, 276-278 (2001).
2. A. V. Zhdanov, E. A. Kolobova, D. A. Varlamov, *et al.*, *Klin. Lab. Diagn.*, No. 11, 33-37 (2000).
3. A. V. Zhdanov, D. F. Kurbanova, M. P. Davydova, *et al.*, *Byull. Eksp. Biol. Med.*, **135**, No. 2, 173-177 (2003).
4. A. V. Zhdanov, L. Yu. Sosulina, D. F. Kurbanova, *et al.*, *Ibid.*, **134**, No. 11, 555-559 (2002).
5. V. P. Smetnik and L. G. Tumilovich, *Noninvasive Gynecology* [in Russian] (1997), pp. 504-526.
6. A. S. Elhafey, J. C. Papadimitriou, M. S. El-Hakim, *et al.*, *Arch. Pathol. Lab. Med.*, **125**, No. 7, 872-879 (2001).
7. M. Leone, C. Costantini, G. Gallo, *et al.*, *Maturitas*, **16**, No. 1, 31-38 (1993).
8. H. M. Morsi, M. P. G. Leers, M. Radespiel-Troger, *et al.*, *Gynecol. Oncol.*, **77**, 11-17 (2000).
9. H. Niikura, H. Sasano, K. Kaga, *et al.*, *Hum. Pathol.*, **27**, No. 3, 282-289 (1996).
10. B. Risberg, K. Karlsson, V. Abeler, *et al.*, *Int. J. Gynecol. Pathol.*, **21**, No. 2, 155-160 (2002).
11. S. Waga and B. Stillman, *Annu. Rev. Biochem.*, **67**, 721-751 (1998).
12. B. Waksmanski, J. Dudkiewicz, and S. Dabrowski, *Wiad. Lek.*, **54**, No. 11-12, 656-661 (2001).