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Bcl-2, BAX, and apoptosis in endometrial hyperplasia after high dose gestagen therapy: A comparison of responses in patients treated with intrauterine levonorgestrel and systemic medroxyprogesterone

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Abstract

Objectives. The aim of the study was to investigate apoptosis as a growth regulatory mechanism of gestagen in endometrial precancers and to compare differences in the apoptotic cascade after high and low dose gestagen regimens.

Method. Pre- and post-treatment paraffin-embedded endometrial hyperplasia specimens from women treated with levonorgestrel intrauterine device ($n = 26$) and women treated with 10 mg medroxyprogesterone for 10 days per cycle ($n = 31$) were examined for changes in the expression of Bcl-2 and BAX and the extent of apoptosis after 3 months of treatment. Immunohistochemical expression in tissue specimens for Bcl-2 and BAX was evaluated by H-score. Average number of apoptotic cells per hundred cells within ten different high power field (40×) was evaluated for each section after in situ apoptosis detection (TUNEL method). A second group of patients with endometrial hyperplasia was examined after 1 week treatment with levonorgestrel IUD ($n = 6$) or medroxyprogesterone ($n = 5$) to determine early effects on expression of Bcl-2 and BAX and the extent of apoptosis.

Results. All the patients in the IUD group ($n = 31$) but only about half of the patients in per oral group (16 of 26) responded to treatment. The glandular reduction in Bcl-2 expression was markedly greater for the IUD patients than for the patients who received oral gestagen. The decrease in BAX expression after IUD treatment was less than the reduction of Bcl-2. Decrease in glandular Bcl-2 after 3 months of treatment was coincident with a significant increase in the measurable amount of apoptosis. In stromal cells, the increase in expression of Bcl-2 and BAX was found after gestagen treatment, the response being much more marked for the IUD group. The non- responders to per oral gestagen had no Bcl-2 expression in stroma after 3 months of therapy whereas an increase was observed for the responders. After 1 week, glandular Bcl-2 expression was significantly reduced after treatment in the IUD group. As for the rest, no changes were detected in either of the groups.

Conclusion. Our results indicate that proteins in the apoptotic cascade are regulated by gestagen therapy in human endometrial precancers. Expression of these proteins is shown to be dependent on administration form and/or type of gestagen. Stromal Bcl-2 expression appears to be a potential biomarker which can separate responders of gestagen treatment from non-responders after oral administration.

Keywords: Endometrial hyperplasia; Apoptosis; Gestagens; Therapy

Introduction

A recently published report showed that local application of an intrauterine device with high-dose gestagens was superior to systemic treatment in women with endometrial hyperplasia [1]. This may be the result of an exposure to

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dose being many-fold higher due to the local application [2]. Although the result seemed favorable, only a few earlier studies with intrauterine gestagen device have been performed [3–5]. Gestagen hormones have a documented antiproliferative effect in the human endometrium in vivo as well as in vitro. Former studies suggest that at least 60% of patients with endometrial hyperplasia will respond to gestagen therapy and various treatment regimens have been given to patients [6–10]. The secretory changes induced by endogenous progesterone in the normal cycling endometrium are accompanied by various molecular processes, among which apoptosis has shown to be of great importance [10,11]. Apoptosis or programmed cell death in the histological normal human endometrium after intrauterine levonorgestrel device has also been demonstrated [11]. Although the information of molecular gestagen influence on apoptosis in the human hyperplastic endometrium is incomplete, the complex process of apoptosis has been studied during the last decades in different tissues and cells. The apoptotic cascade has shown to be regulated by extracellular signals or controlled by intracellular autonomous genetic programs [12]. The initial morphological characteristic of apoptosis is cell shrinkage. This observation is in agreement with a former study describing a mean reduction in glandular cell nuclei of the hyperplastic endometrium after gestagen therapy [1]. The controlled and actively regulated cascade of apoptosis leads to cell death by internucleosomal DNA cleavage [13]. The ultimate end-points of apoptosis (apoptotic bodies) as well as the individual proteins of the cascade can be detected by several techniques, like immunohistochemistry and in situ hybridization [10,11,14].

One of the most studied anti-apoptotic proteins is Bcl-2, shown to protect cells from apoptosis by regulating mitochondrial membrane function [15,16]. Another member of the Bcl-2 family, the BAX protein, increases the apoptotic susceptibility of cells in several organs [16]. To further elucidate the biologic sensitivity of apoptotic proteins to gestagens in endometrial hyperplasia, we measured the expression of Bcl-2 and BAX proteins and apoptotic cells in hyperplastic endometrium treated with different gestagen regimens prior to and after 3 months of therapy. In a second group of patients, the effect after 1 week of treatment was determined to detect early effects on Bcl-2 and BAX expression, and apoptotic cells. The changes in endometrial biopsies from patients treated with intrauterine high dose gestagen device were compared to those from patients who received low dose systemic therapy.

Materials and methods

Morphometry/D-score measurement

The objective prognostic image analysis algorithm, D-score, was performed for all hyperplasia specimens to decide on individual prognostic risk of cancer development and to exclude the high-risk patients from the study. In the original computerized morphometrical analysis study on endometrial hyperplasia, a total of 10 nuclear features and 12 architectural features were analyzed. Using a linear stepwise regression analysis and discriminant analysis, three of these quantitative features were selected as having significant independent prognostic value and were combined into the formula called D-score, as follows:

$$\begin{aligned} \text{D} - \text{score} &= 0.6229 + 0.0439 \times (\text{volume percentage stroma}) \\ &\quad - 3.9934 \times \text{Ln}(\text{standard deviation shortest nuclear axis}) \\ &\quad - 0.1592 \times (\text{outer surface density glands}), \end{aligned}$$

where Ln stands for natural logarithm. The combination of these features had a better discriminating power for cancer progression-or-not than any of many single or other combinations of features analyzed. Of the three different criteria of the D-score, it has been shown before that the volume percentage of stroma and the standard deviation of the shortest nuclear axis are the best discriminators of cancer in the follow-up, although the outer surface density of glands still adds power to the discriminating ability between no-progression and progression cases. The measurements were performed with a Q-PRODIT image analysis system (version 6.1; Leica, Cambridge, UK) described by Baak and coworkers [17].

The prognostic D-score offers the possibility to select patients with high risk (D-score <0), very low risk (D-score >1), and intermediate risk (D-score 0–1) of cancer development. The prognostic value of the D-score has now been confirmed in a number of retro- and prospective studies performed in Europe and the USA, and the stratification by D-score into different prognostic groups gives a more controlled and accurate test situation for investigation of intrauterine gestagen treatment regimens [18–20]. D-score measurements for hyperplasia have been implemented as routine investigation in northern Norway. The initial diagnosis of hyperplasia and D-score was subsequently followed by control specimens for all patients.

Patients

Two different groups were investigated. In the main study group, the effect of gestagens was determined after 3 months. The effect in the second group was assessed after 1 week. In the main group, a total of 57 patients received hormonal treatment for endometrial hyperplasia, of these 26 patients had a gestagen intrauterine device, 31 received per oral therapy with medroxyprogesterone acetate. All the 26 patients in the levonorgestrel intrauterine device (IUD releasing 20 μg of levonorgestrel per day) group were included in the study after informed consent since this treatment for hyperplasia is not routinely given in Norway. The patients were referred to the University Hospital of