Available online at www.sciencedirect.com

SCIENCE DIRECT

Gynecologic Oncology 97 (2005) 483 – 488

Gynecologic Oncology

www.elsevier.com/locate/ygyno

Estrogen and progesterone receptors and cyclooxygenase-2 expression in endometrial cancer, endometrial hyperplasia, and normal endometrium

Francisco J. Orejuela[a], Lois M. Ramondetta[b,*], Judith Smith[b], Jubilee Brown[b], Luciano B. Lemos[c], Yang Li[b], Lisa M. Hollier[a]

[a]*Departments of Obstetrics, Gynecology and Reproductive Sciences, The University of Texas Health Science Center, Houston, TX 77025, USA*
[b]*Department of Gynecologic Oncology, The University of Texas M. D. Anderson Cancer Center Unit 440, 1515 Holcombe Boulevard, Houston, TX 77030, USA*
[c]*Department of Pathology, Lyndon B. Johnson Hospital, Houston, TX 77026, USA*

Received 13 October 2004
Available online 17 March 2005

Abstract

Objectives. To determine whether cyclooxygenase-2 (COX-2) expression is seen in endometrial cancer, endometrial hyperplasia, and normal endometria and whether it correlates with expression of estrogen and progesterone receptors.

Methods. The study was a retrospective, IRB-approved analysis of biopsy samples from 14 patients with endometrial adenocarcinoma, 19 with endometrial hyperplasias, and 10 with normal endometrium. Excluded were samples from women with a history of pelvic radiation, NSAID use, or treatment with hormones during previous year. Immunohistochemical analyses were performed on formalin-fixed, paraffin-embedded tissues. Expression of COX-2, estrogen and progesterone receptors were scored according to the proportion of positive-staining cells: 1^+, <10%; 2^+, 10–50%; and 3^+, >50%. A score $\geq 2^+$ was considered positive. Fisher's exact test and analysis of variance were used to compare proportions and continuous variables, respectively.

Results. Overexpression of COX-2 was seen in 4 (29%) of the endometrial cancers, 6 (32%) of the endometrial hyperplasia, and 4 (20%) of the normal endometria. These differences were not statistically significant ($P = 0.90$). No COX-2 expression was found in stromal tissue. Of 14 endometrial cancers, 7 (50%) expressed any COX-2, with 4 (29%) having an expression score of $\geq 2^+$. Of 19 endometrial hyperplasias, 11 (58%) expressed any COX-2; with 6 (32%) having a score of $\geq 2^+$. All 10 normal endometria showed only 1^+ expression. No significant differences were detected in COX-2 expression by grade or stage of cancer. Although 100% and 95% of both hyperplasia and normal endometrium samples expressed in estrogen and progesterone receptors, respectively, only 71% and 79% of endometrial cancers expressed estrogen and progesterone receptors ($P = 0.01$). A nonparametric trend was performed to detect a relationship, between COX-2 and estrogen receptor or progesterone receptor expression; no significant trend was found.

Conclusions. In this study, the immunohistochemical analysis showed a trend toward increased COX-2 expression in endometrial cancer and hyperplasia compared to normal endometria. A larger sample size is needed to confirm these results. The increased COX-2 expression in hyperplasia may signify an early step in carcinogenesis. These findings may represent an important treatment opportunity for synergism in the hormonal therapy of endometrial cancer.

Keywords: COX-2; Endometrial cancer; Estrogen receptor; Progesterone receptor

Introduction

Endometrial cancer is the most common gynecologic malignancy and the fourth most common cancer in women in the United States. Since 1987, the mortality rate for endometrial adenocarcinoma has increased by 50% [1]. The etiology of adenocarcinoma of the endometrium is not completely understood. The risk for developing type I endometrial adenocarcinoma is proportional to the degree of abnormal endometrial proliferation (i.e., simple vs. complex) as well as the presence of cellular atypia.

* Corresponding author. Fax: +1 713 792 7586.
E-mail address: lramonde@mdanderson.org (L.M. Ramondetta).

0090-8258/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.ygyno.2005.02.010

Among the many identified risk factors for type I endometrial cancer, obesity is the most important, increasing the risk 3–10 times more than that for women at their ideal body weight [2,3]. Obesity may increase this risk as a result of elevated levels of estradiol conversion in peripheral adipose tissue or as a result of the direct carcinogenic effects of dietary N-6 polyunsaturated fatty acids, which are metabolized via cyclooxygenase (COX) and lipooxygenase enzymes [4]. There is evidence that byproducts of these enzymes promote prostate and breast cancers, both of which are hormonally influenced tumors [5,6]. Although this direct association has not yet been demonstrated in endometrial cancer, animal fats are known to be associated with increased endometrial cancer risk [4].

Several recent studies have suggested that COX is a therapeutic target for cancer prevention. This hypothesis originated in epidemiologic studies showing a lower risk of colon, prostate, and bladder cancers among users nonsteroidal anti-inflammatory drugs (NSAIDs) [7–9]. Increased COX-2 expression has been identified in many types of malignancies, including colon [9], cervical [10] and breast [11,12], prostate [13], gastric [14], and bladder [15] cancers.

Hormonal control of COX-1 and COX-2 has been suggested in the literature but not fully explored. In neuroblastoma cell lines, COX-1 has been found to be upregulated by dexamethasone, hydrocortisone, and aldosterone. Estrogen did not alter this upregulation; however, mifepristone was able to reverse the effects of the glucocorticoids, suggesting that the control in neuroblastoma cell lines is related to glucocorticosteroid receptors and not to estrogen [16]. Morisset et al. [17] showed that 17-β estradiol inhibits COX-2 mRNA expression in cultured bovine chondrocytes and that it is as potent as dexamethasone in inhibiting both COX-2 mRNA expression and prostaglandin production. In their study, when the cells were treated with tamoxifen, thus blocking the estrogen receptor, the inhibition effect was lost, suggesting that the active site of inhibition occurred at the estrogen receptor and not the glucocorticoid receptor. Zhou et al. identified an alternative mechanism of hormonal control that relates to the interaction of human chronic gonadotropin and luteinizing hormone that promotes morphologic as well as functional differentiation of endometrial stromal cells into decidua. These morphologic changes are mediated by prostaglandin E2, a byproduct of the COX-2 pathway [18].

Given the evidence that prostaglandin production may be hormonally mediated, we hypothesized that COX-2 expression in endometrial cancer is also hormonally mediated and that this hormonal action may partially explain the utility of progesterones in treating endometrial cancer and endometrial hyperplasia. To more fully understand the pathogenesis of endometrial cancer and the mechanism of prognostic molecular indicators, we investigated whether the level of expression of COX-2 in endometrial cancer, endometrial hyperplasia, normal endometrial controls, and if COX-2 expression is correlated with the expression of estrogen receptors and progesterone receptors.

Materials and methods

Patient samples

This retrospective study received approval from the institutional review boards of The University of Texas M. D. Anderson Cancer Center and Lyndon B. Johnson General Hospital in Houston, Texas.

We reviewed endometrial biopsy samples from 14 women with endometrial adenocarcinoma, 19 with endometrial hyperplasia, and 10 with normal endometria; the normal samples were selected from various phases of the menstrual cycle. All samples were collected between January 1, 1999, and December 31, 2003. Exclusion criteria included history of pelvic radiation, use of NSAIDs, and hormone treatment during the previous year. Medical records were reviewed to ascertain age, parity, menopausal status, medical history, history of radiotherapy or chemotherapy, body mass index, and history of hormone replacement therapy.

Immunohistochemical analysis

Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded tissue sections (4 μm) using the labeled streptavidin–biotin method (LASB kit; Dako Corp., Kyoto, Japan). Routine deparaffinization from xylene to 95% alcohol and rehydration were performed. The tissue samples were subsequently treated with 0.3% hydrogen peroxide in methanol for 30 min. For antigen retrieval, the slides were microwaved for 10 min. Primary antibodies were incubated overnight at 4°C. The primary antibodies used were rabbit polyclonal antibody 18515 (Immunobiological Laboratories, Gunma, Japan) at a 1:50 dilution for COX-2 and monoclonal antibodies (Novocastra Labs, Newcastle—Tyne, UK) for the estrogen and progesterone receptors. Sections were incubated with a biotin-labeled secondary antibody for 20 min, and peroxidase-conjugated streptavidin was added for 20 min. The bound antibody complexes were stained for 10 min with 0.05% diaminobenzidine. The sections were then counterstained with methyl green, dehydrated, and mounted.

The COX-2, estrogen receptor and progesterone receptor expression levels determined by the immunohistochemical analyses were scored according to the proportion of positive cells as follows: 0, no staining; 1, less than 10%; 2, 10–50%; and 3, >50%. A result was considered positive if the score was $\geq$2 ($\geq$10%). A gynecologic pathologist (R.R.B.), who was blinded to the patients' clinicopathologic factor, reviewed the stained slides.