Increased Circulating Levels of Insulin-Like Growth Factor-I and Decreased Circulating Levels of Insulin-Like Growth Factor Binding Protein-1 in Postmenopausal Women with Endometrial Cancer

TAKUYA AYABE, OSAMU TSUTSUMI, HIDEKI SAKAI, HIROYUKI YOSHIKAWA, TETSU YANO, FUMIHIKO KURIMOTO*, AND YUJI TAKETANI

Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, Tokyo 113, and
*Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo 174, Japan

Abstract. In human endometrium insulin-like growth factor binding protein (IGFBP)-1 inhibits the mitogenic action of insulin-like growth factor (IGF)-I by inhibiting the binding of IGF-I to its receptor. Our purpose was to compare circulating levels of IGF-I and IGFBP-1 in women with and without endometrial cancer. We assessed circulating levels of IGF-I and IGFBP-1 and IGFBP-3 in 23 patients with endometrial cancer, 11 patients with uterine cervical cancer and 27 healthy control women. The mean circulating level of IGF-I decreased significantly following menopause but was not correlated with age in the control group. The body mass index was significantly higher in the endometrial cancer group than in the control group. Analysis of covariance showed that even after the data were adjusted to eliminate the influence of the body mass index, the circulating IGF-I concentration was higher in postmenopausal endometrial cancer patients than in postmenopausal control subjects. The mean circulating level of IGFBP-1 was significantly lower in postmenopausal cancer patients than in postmenopausal control subjects. There were no significant differences in the serum levels of IGF-I and IGFBP-1 in the patients with cervical cancer and the control group. In conclusion, an increased circulating concentration of IGF-I and a decreased circulating concentration of IGFBP-1 are associated with endometrial cancer especially in postmenopausal women.

Key words: Insulin-like growth factor-I, Insulin-like growth factor binding protein-1, Endometrial cancer

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INSULIN-like growth factor (IGF)-I and IGF-II stimulate cell growth and differentiation in a variety of tissues and cell lines. Although circulating IGF-I and IGF-II are synthesized mainly by the liver, IGFs are also synthesized locally and act as autocrine/paracrine growth factors. IGF-I and IGF-II messenger RNAs (mRNAs) have been detected in the uterus of humans, and IGF binding sites have been localized in the myometrium and the endometrium [1]. IGFs are believed to be involved in the regulation of estrogen-induced cellular proliferation in the normal endometrium [1]. IGF receptors are expressed in endometrial cancer tissue where IGFs act as potent mitogens [2].

IGFs bind to specific proteins termed IGF-binding proteins (IGFBPs) in serum. To date six IGFBPs have been cloned and sequenced [3]. The biological actions of IGFs are modified by IGFBPs, which inhibit or enhance the effects of IGFs at the cellular level. IGFBP-1 is synthesized in the liver
and in decidualized endometrium and predominates in human endometrium [1]. IGF-I has a similar affinity for IGFBP-1 and type I IGF receptors [4]. IGFBP-1 inhibits the binding of IGF-I to its endometrial IGF receptors [5], thereby inhibiting the mitogenic action of IGF-I in the endometrium [6]. IGFBP-3, whose synthesis is regulated by GH [7], is a major binding protein in serum: approximately 90% of IGF-I in serum binds to IGFBP-3, with the remainder binding to IGFBP-2 or IGFBP-1 [8].

Although estrogen increases the risk of endometrial cancer, this cancer is common in postmenopausal women. The mechanism of action of estrogen is not completely understood, but it is believed to increase the risk of endometrial cancer at least in part by inducing the secretion of autocrine/paracrine IGFs and the expression of IGF receptors. Growth factors are believed to be produced independently of systemic hormones and to contribute to the development of endometrial cancer in postmenopausal women. In a previous study circulating levels of IGF-I were lower in patients with endometrial cancer compared with the control group, but there was no difference between groups in the circulating levels of IGFBP-1 [9]. In the present study, we measured circulating levels of IGF-I and IGFBPs in women with and without endometrial cancer, and have shown that the mean circulating level of IGF-I was significantly higher and the mean circulating level of IGFBP-1 was significantly lower in postmenopausal endometrial cancer patients than in postmenopausal control subjects.

Materials and Methods

Subjects

We studied 23 Japanese women with primary endometrial adenocarcinoma (30 to 74 years old) including 5 premenopausal and 18 postmenopausal women. Stage I disease was present in 11 patients, stage II in 3 patients, and stage III in 9 patients based on the International Federation of Gynecology and Obstetrics staging, 1988. The histopathologic diagnoses were adenocarcinoma type in 22 patients and adenosquamous type in 1 patient. Histologic differentiation was classified as follows: G1: 10 patients; G2: 10 patients; and G3: 3 patients. None of the patients had any evidence of diabetes mellitus, impaired hepatic function, renal failure, or systemic diseases.

We also studied a group of 11 Japanese women with uterine cervical cancer (41 to 66 years old), including 4 premenopausal and 7 postmenopausal women. The control group consisted of 27 apparently healthy women (22 to 75 years old) including 19 premenopausal and 8 postmenopausal women. None of these subjects were receiving any hormones or other medications.

Body mass index (BMI) was calculated according to the following formula: body weight (kg) divided by the square of height (m²).

Informed consent was obtained from all subjects before the initiation of the study. The study protocol was approved by the hospital ethics committee.

Sampling

Baseline blood samples were collected after an overnight fast for determination of IGF-I, IGFBP-1 and IGFBP-3 levels. Serum was separated by centrifugation at 4 °C and stored at −20 °C until assayed.

Assays

For measurement of IGF-I, serum samples were extracted by the acid-ethanol method according to the method of Daughaday [10, 11]. IGF-I was measured with the Somatomedin-C RIA Kit (Ciba-Corning, Tokyo, Japan) in which the tracer was prepared from recombinant IGF-I. The intra- and inter-assay coefficients of variation were 6.8 to 14.8% and 4.3 to 14.7%, respectively.

IGFBP-1 levels were measured by an immunoradiometric assay using two monoclonal antibodies to IGFBP-1 (Mab6303 and Mab6305) according to a previously described method [12, 13]. The intra- and inter-assay coefficients of variation were 2.87 to 4.84% and 5.61 to 15.4%, respectively.

IGFBP-3 levels were measured with the DSL-6700 IGFBP-3 RIA Kit (Diagnostic Systems Laboratories, Webster, Texas, USA). The intra- and inter-assay coefficients of variation were 4.83 to 4.85% and 4.65 to 7.25%, respectively.
Statistical analyses

Results are expressed as the mean ± SEM. Between group comparisons were made by the Student's t-test for unpaired data. The relationship between selected variables was determined by linear regression analysis. Two-way factorial analysis of variance was performed to estimate the effects of two categories on a variable. Analysis of covariance was used to revise the difference of covariate between two categories.

Results

Circulating concentrations of IGF-I and menopause

In both the control group and the endometrial cancer group, the circulating concentration of IGF-I was not correlated with age when the premenopausal or the postmenopausal women were analyzed separately. The mean circulating level of IGF-I was significantly lower in postmenopausal women than in premenopausal women in the control group, but there was no significant difference in the IGF-I levels between postmenopausal and premenopausal women in the endometrial cancer group (Table 1). Thus, among postmenopausal women, the mean circulating level of IGF-I was significantly higher in the endometrial cancer group than in the control group.

Relationship between the circulating concentration of IGF-I and BMI

The circulating levels of IGF-I were not correlated with the BMI in premenopausal control subjects or endometrial cancer patients, but there was a significant positive correlation between the IGF-I concentration and BMI in endometrial cancer patients (Fig. 1; R=0.568, P=0.0139).

The BMI was significantly higher in postmenopausal endometrial cancer patients than in postmenopausal control subjects (23.1 ± 0.4 kg/m² vs. 21.6 ± 0.6 kg/m², P<0.05). Since the category of endometrial cancer/control group did not have statistical interactive effects on the category of BMI, analysis of covariance could be employed to reassess the difference in BMI between endometrial cancer patients and control subjects so that the influence of BMI could be eliminated. Analysis of covariance showed that the circulating level of IGF-I was higher in postmenopausal endometrial cancer patients than in the control subjects even after the data were adjusted for BMI (P<0.01).

Circulating concentration of IGFBP-1

In the control group or the endometrial cancer group, the circulating concentration of IGFBP-1 was not correlated with age when the premenopausal or the postmenopausal women were analyzed separately. The mean circulating level of IGFBP-1 was significantly higher in the postmenopausal control group than in the premenopausal control group (Table 1), but there was no significant difference between the postmenopausal and the premenopausal endometrial cancer patients, so that among postmenopausal women, the circulating level of IGFBP-1 was significantly lower in endometrial cancer patients than in the control group.

Neither the BMI nor the IGF-I concentration was correlated with the IGFBP-1 concentration in either the endometrial cancer patients or the control subjects (Fig. 2).

Table 1. Circulating levels of insulin-like growth factor (IGF)-I, insulin-like growth factor binding protein (IGFBP)-1, and IGFBP-3

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
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<tbody>
<tr>
<td></td>
<td>control (n=19) cervical cancer (n=4) endometrial cancer (n=5)</td>
<td>control (n=8) cervical cancer (n=7) endometrial cancer (n=18)</td>
</tr>
<tr>
<td>IGF-I (ng/mL)</td>
<td>183.6 ± 13.2 196.3 ± 54.5 174.8 ± 26.9</td>
<td>102.5 ± 8.8* 94.7 ± 13.1 155.2 ± 6.9**</td>
</tr>
<tr>
<td>IGFBP-1 (ng/mL)</td>
<td>7.61 ± 1.53  3.90 ± 2.65  5.42 ± 3.92</td>
<td>16.81 ± 4.71* 15.91 ± 6.03  5.64 ± 2.01**</td>
</tr>
<tr>
<td>IGFBP-3 (pg/mL)</td>
<td>2.81 ± 0.15  2.95 ± 0.32  2.85 ± 0.22</td>
<td>2.76 ± 0.28  2.55 ± 0.21  2.57 ± 0.15</td>
</tr>
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Values are the mean ± SEM. *: P<0.001, +: P<0.05 vs. premenopausal control, **: P<0.001, ++: P<0.05 vs. postmenopausal control.
Circulating concentration of IGFBP-3

The circulating level of IGFBP-3 was not correlated with age, menopausal status, or BMI.

There was no significant difference in the IGFBP-3 levels between the control group and the endometrial cancer group (Table 1).

Stage and differentiation of endometrial cancer

The mean circulating levels of IGF-I, IGFBP-1 and IGFBP-3 were not related to the stage or histologic differentiation of endometrial cancer.

Circulating concentrations of IGF-I, IGFBP-1 and IGFBP-3 in the uterine cervical cancer patients

There were no significant differences in the serum concentrations of IGF-I, IGFBP-1 and IGFBP-3 between the patients with cervical cancer and the healthy control group (Table 1).

Discussion

The mean circulating level of IGF-I was significantly higher and the mean circulating level of IGFBP-1 was significantly lower in postmenopausal endometrial cancer patients than in postmenopausal control subjects or cervical cancer patients. These results are inconsistent with the findings of a previous study in Finnish women [9]. The discrepancy may be due, in part, to racial differences: Japanese women are smaller and there may be differences in the metabolism of carbohydrate and estrogen between Japanese and Finnish women. In addition, the levels of coexisting IGFBPs may have affected IGF-I values because acid-ethanol extraction, which was used in both studies, does not completely eliminate IGFBPs from samples [14]. Mohan and Baylink [14] have reported the effectiveness of acid-ethanol extraction as a means of eliminating the influence of IGFBPs on the measurement of IGF-I, and suggested that the difference between the IGF-I levels can be considered as significant if the magnitude of the difference is greater than 30%. In the present study, the difference in the IGF-I levels between endometrial cancer patients and the control group was greater than 50%, suggesting that the difference could not be explained as a methodological error.

The circulating concentration of IGF-I are reported to decrease with age in both sexes [15–17]. In the present study, the mean circulating...
level of IGF-I decreased following menopause but was not correlated with age when the premenopausal and the postmenopausal group were analyzed separately. This finding is consistent with a recent study by Romagnoli et al. [18] who found that the serum level of IGF-I decreased immediately after menopause but was not correlated with the interval after menopause. Weissberger et al. [19] have suggested that estrogen increases the circulating level of IGF-I, and estrogen has been found to stimulate the expression of both IGF and IGF-receptor mRNA in vitro in human endometrial cells and endometrial cancer cells [20, 21]. The present results provide further support for a relationship between estrogen and the IGF-I level.

In postmenopausal women, because most of the estrogen is produced by peripheral aromatization of androstenedione in fatty tissue, the amount of circulating estrogen in postmenopausal women is supposed to be correlated with the BMI, an index of the total amount of fatty tissue. We found a positive correlation between the circulating concentration of IGF-I and BMI in postmenopausal women in the present study, which is consistent with the reported relationship between estrogen level and IGF-I production.

The BMI was higher in postmenopausal cancer patients than in the control group in the present study. It is possible to consider that the increased level of IGF-I in postmenopausal cancer patients may have been due to the higher BMI in this subgroup compared with the control group. Analysis of covariance showed, however, that the IGF-I level was significantly higher in endometrial cancer patients than in the control group even when BMI was matched. These findings suggest that an increased circulating level of IGF-I may be a risk factor for endometrial cancer. It is possible, however, that the higher circulating level of IGF-I reflected unknown predisposing factors for endometrial cancer. The number of IGF-I receptors is higher in endometrial cancer tissue than in the normal endometrium [2], so that once endometrial cancer develops, the increase in receptors may further enhance the biological actions of IGF-I, promoting cancer growth.

A reverse transcription-polymerase chain reaction study showed that IGFBP-1 mRNA was undetectable or minimally expressed in endometrial cancer tissue compared with normal endometrial tissue from premenopausal women [22]. IGFBP-1 inhibits the binding of IGF-1 to its receptors on the endometrium [5], thereby inhibiting the biological actions of IGF-I in the endometrium [6]. The mean circulating level of IGFBP-1 was significantly lower in postmenopausal endometrial cancer patients than in control subjects in the present study. A decrease in the IGFBP-1 level may further enhance the actions of IGF-I, thus stimulating the proliferation of endometrial cells and resulting in the development of endometrial cancer although IGFBP-3 present in large amounts may also play an unknown role in the regulation of IGF-1 actions.

A possible involvement of insulin action on IGFBP-1 production and modification of IGF-I activity by IGFBP-3 are to be elucidated, but this has not yet been done. The roles of circulating IGF-I and IGFBP-1 in the pathogenesis of endometrial cancer are also yet to be determined. The present findings at least suggest that a high circulating concentration of IGF-I and/or a low circulating concentration of IGFBP-1 may be risk factors for endometrial cancer in postmenopausal women. Further investigation is needed to clarify the relation between the IGF system and the pathogenesis of endometrial cancer. Measurement of circulating levels of IGF-I and IGFBP-1 may be a screening test for endometrial cancer, especially in postmenopausal women.

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