J. steroid Biochem. Vol. 36, No. 6, pp. 523–526, 1990
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0022-4731/90 $3.00 + 0.00
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OVARIAN FUNCTION IN PREMENOPAUSAL WOMEN AFFECTED BY BREAST CANCER: THE MEASUREMENT OF GLUCURONOCONJUGATE METABOLITES OF 17β-ESTRADIOL AND PROGESTERONE THROUGHOUT ONE ENTIRE MENSTRUAL CYCLE

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(*Received* 2 *May* 1989; *received for publication* 24 *May* 1990)

Summary—For many years, hypersecretion of estrogens has been suspected of being one of the major risk factors of breast cancer for premenopausal women.

Seventeen premenopausal women, who had undergone lumpectomy because of breast cancer (T1a No Mo) 3 yr before entering the study, were compared to 9 normal women of similar age, parity and body weight. A chemiluminescent method was used for the determination of estrone-3-glucuronide (E1-3G) and pregnanediol-3-glucuronide (Pd-3G) in early morning urine samples collected for an entire menstrual cycle of each of the 26 subjects.

During the follicular phase, no significant differences in E1-3G and/or Pd-3G excretion were found between the two groups. During the luteal phase the E1-3G/Pd-3G ratio in the early, middle and late luteal phase had significantly increased in the women with breast cancer, in spite of normal Pd-3G excretion. Therefore, the measurement of glucuronoconjugate metabolites of ovarian hormones in overnight urine might be conveniently applied to the study of ovarian function in subjects with breast cancer. Furthermore, the results of this study may indicate that an estrogen/progesterone imbalance is an additional risk factor for the premenopausal breast cancer patient.

INTRODUCTION

The hypersecretion of estrogens and or an altered 17β-estradiol/progesterone (17βE2/P) balance may be a risk factor for breast cancer in menopausal women [1, 2].

However, scientific demonstration of this hypothesis, using blood or urine samples, has been impeded by several practical problems: (a) the hormonal pattern of the menstrual cycle in any single woman may differ from one to another; (b) the collection of daily blood samples during an entire menstrual cycle is often as unacceptable to the cancer patient as it is to the normal control; (c) the pulsatile secretion of steroid hormones increases intra-subject and inter-subject variability, when a single daily blood sample is taken [3]; (d) 24 h urine sample collection throughout one entire menstrual cycle is tedious; and (e) when urinary ovarian hormone metabolites are measured using hydrolytic procedures, the results are not always reliable [4]. The measurement of glucuronoconjugates of ovarian hormones in diluted overnight urine samples has been recently applied to the study of ovarian function [5–7]. Therefore, glucuronoconjugate metabolites of 17βE2 (estrone-3-glucuronide; E1-3G) and P (pregnanediol-3-glucuronide; Pd-3G), in diluted urine, were measured in order to study ovarian secretion in premenopausal women affected by breast cancer and in normal women of similar age.

MATERIALS AND METHODS

Subjects

Patients. Seventeen premenopausal women (age 35–44 yr; mean ± SD: 40.64 ± 3.52 yr) who had undergone conservative surgery for T1a No Mo breast cancer [8], were investigated. Each subject had a history of normal menstrual cycles. To avoid any possible effects of acute stress on ovarian function which may accompany knowledge of the disease, only those women who had had surgery at least 3 yr before the start of the study (range: 3–5 yr; mean ± SD: 3.7 ± 0.96 yr) were considered. No subject showed any evidence of hepatic or renal disease. None had received adjuvant hormonal therapy and/or chemotherapy after surgery. This disease free interval was chosen to avoid possible interference of estrogen

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Table 1. Area and mean values of E1-3G and Pd-3G in definite time intervals of the menstrual cycle in patients affected by breast cancer and normal sujects

	Breast cancer ($n = 17$)	Normal subjects ($n = 9$)
	E1-3G (ng)	
EFP	612.5 ± 340.2	455.1 ± 294.1 ns
LFP	10076.8 ± 3748.0	8721.8 ± 3053.4 ns
LP	16275.8 ± 8004.6	10958.8 ± 4084.6 ns
	Pd-3G (μg)	
FP	35.4 ± 28.4	27.0 ± 8.1 ns
LP	1961.8 ± 872	2598.7 ± 890 ns
	Ratio E1-3G/Pd-3G	
FP	56.2 ± 34.1	39.3 ± 25.5 ns
LP	15.2 ± 10.09	6.2 ± 3.4
		$P < 0.005$

Capital letter = area values. Small letter = mean values (area values/days of the interval). ns: no statistically significant difference. Values are expressed as mean ± standard deviation (SD).

metabolism by the tumor itself [9], even though the presence of micrometastases could not be excluded. Therefore, the Pd-3G and E1-3G excretion rate in such patients was considered the expression of basal ovarian function.

Normal subjects. Nine healthy volunteers (age range: 31–43 yr; mean ± SD: 38.6 ± 4.3 yr) were investigated. They had normal ovulatory menstrual cycles and were not suffering from any kidney, hepatic and/or endocrine disease.

Normal subjects were comparable for age, parity and body weight with patients affected by breast cancer. Hormonal contraception was not used in the last 6 months. However, no precise information as to the type of contraceptive method use by the 26 subjects was available.

Urine sample collection

Early morning urine samples were collected daily throughout an entire menstrual cycle, as described by Pazzagli *et al.*[7]. Urine collection was performed at home. Hours of sleep and the urinary volume voided in the early morning were recorded daily by each subject on printed forms. In order to standardize the volume of glomerular filtrate during the night and reduce the variability in renal function, all the women drank a fixed amount of water in the evening after dinner (5 ml/kg body wt). Irregularities in urine collection were reported and the entire cycle under investigation was eliminated from the study. No complaints about this procedure were made by any subject.

Assay methods

Chemiluminescent immunoassay methods were used to determine the concentration of E1-3G and Pd-3G. Monoclonal antibodies and chemiluminescent tracers were employed, as previously described by Eshhar *et al.*[10] and Lindner *et al.*[5], to monitor immunological reaction. Briefly, 0.1 ml of a diluted urine sample (1:100 dilution with assay buffer) or 0.1 ml of the standard solution was incubated overnight at 4°C in the presence of both 0.1 ml of the

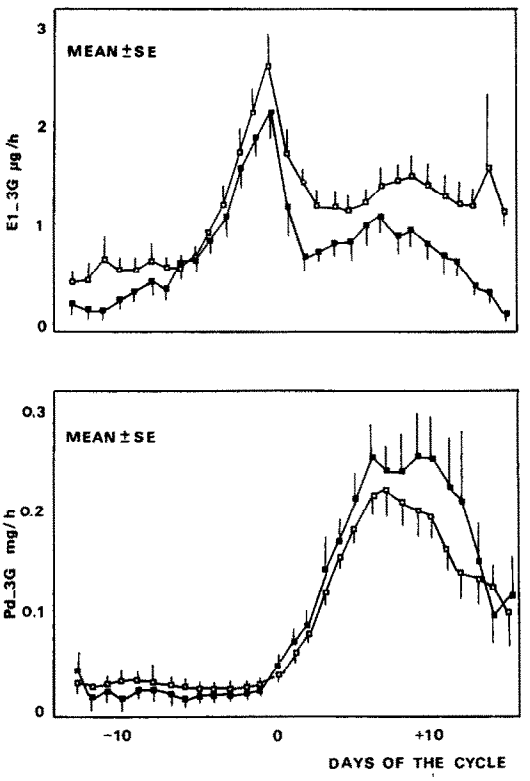

Fig. 1. Urinary profiles of E1-3G and Pd-3G (mean ± SE) in 9 normal women (filled symbols) and in 17 premenopausal women affected by breast cancer (open symbols). The hormonal values are aligned to the LH peak.

chemiluminescent tracer (E1-3G-aminobutylethylisoluminol or Pd-3G-aminobutylethylisoluminol) and 0.1 ml of specific monoclonal antiserum. A dextran-coated charcoal suspension (2.0 mg/tube, 0.2 ml) was then used to separate the bound from the free fractions. Light emission of the supernatant was induced by using a hydrogen peroxide/microperoxidase system and measured using an automatic luminometer.

Urinary luteinizing hormone (LH) was measured using an enzyme immunoassay detected by a chemiluminescent reaction (Amerlite LH; Amersham, England).

Hormonal concentrations were expressed as mass/h, calculated according to Pazzagli *et al.*[7]. This approach allows for reduced intra-subject variability as compared to other approaches, i.e. the mass per gram of creatinine or mass per liter of urine [11].

The intra- and inter-assay coefficients of variation were calculated for Pd-3G: they were 10.7 and 15.7%, respectively. Coefficients of variation for E1-3G were 8.3 and 10.7%, respectively.

Calculations

Hormonal concentrations of E1-3G and Pd-3G are expressed as mass/h. For each subject, they were aligned to the day of LH peak and used for computation of the mean and standard error (SE). Comparison of the hormone levels of the patients affected by breast cancer and those of the normal

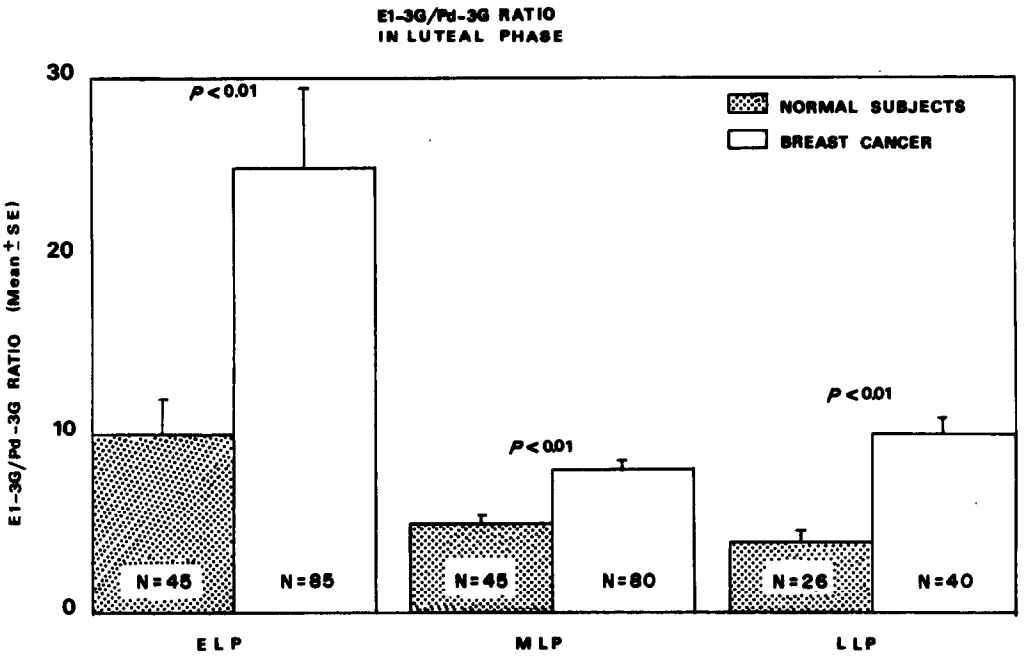

Fig. 2. The mean value of E1-3G/Pd-3G ratio is significantly higher in the patients affected by breast cancer when evaluated in early luteal phase (ELP) (from day +1 to +5), in middle luteal phase (MLP) (from day +6 to +10) or in late luteal phase (LLP) (from day +11 to +14).

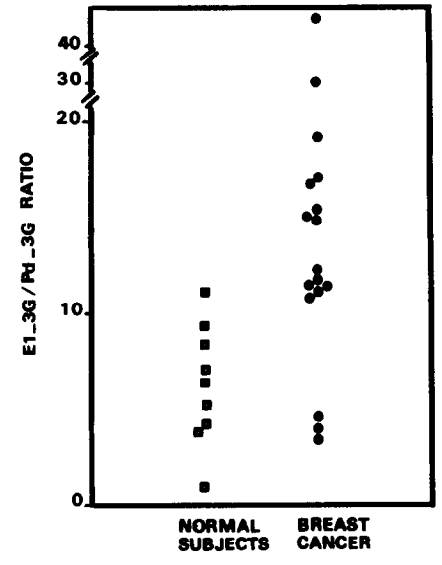

Fig. 3. E1-3G/Pd-3G ratio in luteal phase of 9 normal subjects and 17 premenopausal patients affected by breast cancer. The mean value of the E1-3G/Pd-3G is significantly higher in breast cancer group ($P < 0.005$). About 40% of the 17 patients evaluated presented a E1-3G/Pd-3G ratio within the normal range.

subjects are reported in Fig. 1. However, because of the wide variability in hormonal secretion, the use of hormonal profiles was not able to point out the differences in ovarian hormonal levels between the two investigated groups [7]. Therefore, as previously described [7], additional parameters were calculated using integrated values of the hormonal data at defined time intervals of the menstrual cycle. During the early follicular phase (EFP; from −12 to −6 days), the late follicular phase (LFP; from −5 to 0 days) and during the luteal phase (LP; from +1 day to end of the cycle) E1-3G excretion was calculated. On the other hand, Pd-3G excretion was calculated only in the follicular phase (FP) and during the LP. The integrated values were calculated for each of these periods, so that total hormonal excretion in those definite time intervals of the menstrual cycle were measured. Furthermore, to study the estrogen/progesterone (E/P) balance, the mean E1-3G/Pd-3G ratio of each subject during the follicular and the luteal phase was calculated.

Differences in mean values, for all the data investigated, were evaluated using Wilcoxon's-test.

RESULTS

No significant differences were found during the FP between the premenopausal women affected by breast cancer and the controls (Table 1). During the LP, no significant reduction in Pd-3G excretion was observed (Table 1, Fig. 1). Therefore, in this study, the women affected by breast cancer were not affected by luteal insufficiency [7]. In addition, although the mean integrated value of E1-3G excretion during LP had increased in all the patients affected by breast cancer (Table 1, Fig. 1), the differences were not significant when compared to controls. On the other hand, the E1-3G/Pd-3G ratio was found to be significantly higher in those patients affected by breast cancer during the early, middle, late luteal phases (Fig. 2). The single value of each E1-3G/Pd-3G ratio during the LP is reported in Fig. 3.

DISCUSSION

The measurement of the glucuronoconjugate metabolites of ovarian hormones in early morning urine could be a suitable procedure for the study of ovarian function in premenopausal women affected by breast cancer.

Urinary sampling is better accepted by the patients than daily blood sampling and it can be performed at home. Moreover, the values of urinary steroids correlate very well with circulating steroids [7] and allow for valid results.

The choice of premenopausal women who had been affected by breast cancer (T1a No Mo), and who had surgical intervention at least 3 yr before the study, should have strongly reduced the effect of psychological stress which can affect ovarian function. Thus, the possible production of estrogens caused by the primitive tumour should have also been reduced.

The study of one cycle per woman is open to criticism since it cannot be considered representative of ovarian function over a 1 yr period. However, many other clinical studies on such diverse subjects as luteal insufficiency, polycystic ovarian syndrome [12, 13] etc., have been performed using similar criteria.

Some recent studies have been performed on two groups of premenopausal women affected by breast cancer, during which blood as well as urinary hormones were measured. In these studies the samples were taken on only one day during the FP and on one day during LP [14, 15]. Since the present study is more complicated in terms of its clinical experimental approach, a lower number of control subjects was recruited. Even using different clinical approaches, an E/P imbalance seems to exist in premenopausal breast cancer patients. Nevertheless, it is noteworthy that, in this study, the E/P ratio during LP had increased in only 60% of the cancer patients, while the ratio remained within the normal range in the other 40% (Fig. 3). The wide overlap shown by both control and cancer patients values was previously observed in a study, in which daily circulating ovarian hormones has been measured in 10 premenopausal women affected by breast cancer [16]. These results strongly suggest that the E/P imbalance cannot be considered as the only risk factor in premenopausal breast cancer.

Acknowledgements—We wish to thank Professor G. Cardona for his invaluable contributions. Dr A. Magini is a fellow of the Associazione Italiana per la Ricerca sul Cancro. This work was supported by a grant from the Italian Research Council (Project No. 87.01450.44).

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