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Effect of natural "micronized" progesterone on the chorionic gonadotropin concentrations in cyst fluids of women with gross cystic breast disease

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Abstract

Gross cystic breast disease (GCBD) is common in women, especially in the age range between 35 and the menopausal years. The present study examined the possible role of progesterone (Pg) in the chorionic gonadotropin (hCG) concentration in GCBD. The breast cyst fluids (BCFs) were drawn by fine needle aspiration between the sixth and the eighth day of the menstrual cycle and twenty days later. On the day of the first aspiration the patient began to take 100 mg of natural micronized Pg orally until the second aspiration. At both times blood samples were also taken. Determinations were done of both BCFs and blood sample using two fully automated chemiluminiscent enzyme immunometric assays. Pg has been demonstrated to induce a significant increment in hCG + free ß-hCG (median, range): 0.27 ng/ml, 0.12–6.24 vs. 1.92 ng/ml, 0.12–423.5; free ß-hCG: 0.11 ng/ml, 0.02–2.40 vs. 0.91 ng/ml, 0.02–58.40 in the BCFs, with no change in the circulating concentrations of the hormone. None of the sera studied presented levels of hCG + free ß-hCG or free ß-hCG above 0.5 ng/ml or 0.1 ng/ml, respectively. The occurrence of hCG or a derivative polypeptide in BCFs, when they are present in high concentrations suggests that this glycoprotein could be synthesized in situ and possibly involved in the pathogenesis of GCBD by the degree of differentiation of breast epithelial cells induced by the hormone.

Keywords: Breast cyst fluids; Progesterone; Chorionic gonadotropin

1. Introduction

GCBD is the most common cause of the generally benign lumps in the breast of women in Western societies, especially in the age range between 35 and the menopausal years. hCG is a glycoprotein (237 aa, 38 kD) composed of two dissimilar subunits: α-hCG (92 aa, 16 kD) and ß-hCG (145 aa, 22 kD), joined non covalently [1,2]. The ß-chain is highly specific for the immunological and biological activity of hCG, but seems to be inactive or to be much less active in the free form [3]; α-hCG has the same peptide sequence as the α-subunits of the pituitary glycoprotein hormones: LH, FSH, and TSH. In contrast, the ß-subunits confer hormone specificity and are dissimilar to these other hormones. The heterodimeric hCG is produced mainly in trophoblastic tissue, and is detected in the serum and urine in normal pregnancies and in trophoblastic and some nontrophoblastic diseases.

Following the pioneering report of Bradlow et al.

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[4], the presence of immunoreactive hCG in breast cyst fluids (BCFs) of women with GCBD has been shown by several investigators [5–9]. In 1986, we demonstrated that "micronized" Pg is absorbable orally [10]. The aim of this study was to investigate the effect of oral administration of Pg on hCG and free ß-hCG levels in both sera and BCFs of women with GCBD.

2. Materials and methods

BCFs of 12 selected pre-menopausal women aged 33–42 (mean 37 years) having only one palpable breast cyst (approximately 2.5–3 cm diameter), according to the Haagensen criterium [11] and confirmed by sonomammography, were studied. None of the patients had been taking oral contraceptives, corticosteroids and not any hormone for at least 2 months, had a negative family history of breast cancer, no evidence of hirsutism and normal weight: body mass index >16 and <26; BMI: [weight (kg)/height $(m)^2$]. After the nature of the study was explained to each woman and the written informed consent was obtained, the study protocol was approved by the Local Bioethical Committee, Hospital Municipal Bernardino Rivadavia, Buenos Aires.

Cyst fluids were drawn by fine needle aspiration between the sixth and the eighth and between the twenty-seventh and the twenty-ninth days of the menstrual cycle. In the first cyst aspiration, only 1 ml of BCF was drawn; the remainder was extracted in the second aspiration done 21 days later. On the day of the first aspiration the patient began to take 100 mg daily of natural "micronized" Pg (Merck-Darmstadt) orally, until the second aspiration. At the same time cyst aspirations were done, the blood samples were also drawn. The BCFs were centrifuged (1500 g) and stored at $-20^{\circ}C$ until assayed. The blood samples were centrifuged, the sera divided into two fractions and immediately stored at $-20^{\circ}C$ pending analysis. To avoid interassay variation, the BCFs and blood samples of each patient were analyzed simultaneously. The biochemical analysis involves the assay of intracystic levels of hCG using two different kits from DPC Diagnostic Products Corporation, Los Angeles, (CA) for all BCFs, and for sera from 12 patients.

Kit 1: Immulite hCG is a fully automated chemiluminescent enzyme immunometric assay that permits the quantitative determination of nicked + nonnicked-hCG + free ß-hCG (from now on we term this hCG + free ß-hCG), using a monoclonal antibody specific for the free ß-hCG subunit and a polyclonal hCG alkaline phosphatase-conjugated. This kit detects ß-hCG whether it is part of the intact molecule or a free ß-subunit. The intra- and inter-assay coefficients of variation were 4.5 and 9.2 and the minimal detectable dose of the assay was 0.12 ng/ml.

Kit 2: The Immulite free ß-hCG is an immunometric assay with a monoclonal antibody specific for nicked + nonnicked free ß-hCG (from now on free ß-hCG) and an alkaline phosphatase-labelled polyclonal goat anti-free ß-hCG. The values for healthy nonpregnant women are expected to be <0.1 ng/ml. (1 ng/ml of free ß-hCG is equivalent to 1 mIU of free ß-hCG in terms of the WHO First International Reference Preparation of Chorionic Gonadotropin Beta Subunit (1st IRP 75/551). The intra and interassay coefficients of variation were less than 5.0% and 9.1%, respectively, and the detection limit of the assay is 0.02 ng/ml A rigorous validation was performed in order to exclude interference by matrix effects. The two kits were validated for the measurement of hCG in BCF by several dilutions of a pool containing five BCFs with high hCG concentrations.

The dose-response curves were essentially parallel to those found for hCG standard, verifying that nonspecific effects were absent (data not shown).

3. Statistical analysis

The statistical difference between hCG + free ß-hCG and free ß-hCG levels before and after progesterone administration were analysed by Wilcoxon's rank sum test for paired data ($p < 0.05$ were considered statistically significant). When the levels were below limits of detectability (<0.12 ng/ml for kit 1 and <0.02 ng/ml for kit 2), these values were used in the analysis of results.

4. Results

Table 1 shows concentrations of hCG + free ß-hCG and free ß-hCG in the BCFs of 12 patients studied, before and after the oral administration of Pg. The differences were statistically significant ($p < 0.011$).

Table 2 shows individual values of hCG + free ß-

Table 1
Concentrations of hCG + free ß-hCG and free ß-hCG (ng/ml) in BCFs of 12 patients studied before and after oral administration of progesterone (100 mg/daily, 20 days)

	hCG + free ß-hCG		free ß-hCG	
	Pre Pg	Post Pg	Pre Pg	Post Pg
Mean (SD)	1.56 (2.28)	48.92 (121.47)	0.46 (0.75)	8.00 (16.70)
Median	0.27	1.92	0.11	0.91
Range	0.12–6.24	0.12–423.5	0.02–2.40	0.02–58.40

hCG and free ß-hCG in BCFs of 12 patients studied. Only 3 of 12 patients presented no detectable hCG and free ß-CG before and after Pg administration.

None of the sera studied presented levels of hCG + free ß-hCG (kit 1) or free ß-CG (kit 2) above 0.5 ng/ml or 0.1 ng/ml, respectively. The present data indicate an important accumulation of hCG in some BCFs during oral progesterone treatment, without any change in plasma circulating levels of hCG. The increase of immunoreactive hCG levels was especially significant in patients 4, 5, 8 and 9.

5. Discussion

GCBD is a common disease in women between 35 and 50 years of age, becoming infrequent after menopause. There have been many poorly substantiated theories about the origin and mechanism of the formation of cysts, all generally related to hormonal activity. Biochemical studies have demonstrated that BCFs frequently contain a wide variety of compounds, for example androgens sulfates, mostly dehydroepiandrosterone-sulfate is accumulated up to concentration 100-fold higher than in plasma [12,13]. Interest in the biochemistry of BCFs has increased in recent years as a probable risk factor for breast cancer, despite some occasional reports minimizing the importance of this area of study [14].

Braunstein et al. [15] have demonstrated that hCG-like substances can be detected in sera of normal men and nonpregnant women, suggesting an extratrophoblastic synthesis site. This observation has been confirmed by other authors suggesting that the synthesis of hCG and α-hCG and ß-subunits could originate in the pituitary [16].

A great number of regulatory substances have been shown to modulate placenta hCG synthesis and secretion, negatively modulated by E2 and positively modulated by Pg [17,18]. The surprising increase of immunoreactive hCG levels in the LQs of women 4, 5, 8 and 9, during Pg treatment, without changes in blood concentrations observed in the present study, suggests that the hormone may be synthesized by the epithelial cells lining the cysts or in the surrounding breast tissue.

Epidemiological and experimental studies have demonstrated that certain modifications in the hormonal environment are able to change the risk of developing breast cancer. For example, the human breast reaches its complete differentiated structures at the end of a full term pregnancy, especially in a young women, significantly reducing the risk of developing breast cancer [19]. Russo et al. [20,21] also demonstrated that hCG administration is able to produce a direct inhibitory effect on the initiation of the neoplastic process through the induction of complete differentiation in the normal breast epithelium.

In a recent work [9], we demonstrated an inverse relationship between epidermal growth factor and hCG in BCF, suggesting that hCG in BCFs may have a protective effect by increasing the degree of refractoriness to malignant transformations. Other investigators support this opinion. Badwe et al. [22] conclude that higher circulating levels of Pg in premenopausal operable patients with axillary nodes were associated with significantly better survival and recently Juricskay et al. [23] found that urinary pregnanediol levels were significantly lower in patients with breast cancer.

We concluded that oral administration of natural Pg appears to induce the hCG production and ß-hCG in the BCFs of some women with GCBD, without demonstrable changes in sera. We hypothetically suggest that both hCG and ß-hCG appear to be synthesized in situ in the epithelium lining the cyst or in the surrounding breast tissue. However, since only small BCFs studied, the present results should be considered preliminary.

Table 2
Values of hCG + free-hCG and free ß-hCG in BCFs of 12 patients studied before and after oral administration of progesterone (100 mg/daily, 20 days)[a]

hCG + free ß-hCG (ng/ml)		Patient no.	Free ß-hCG (ng/ml)	
Pre Pg	Post Pg		Pre Pg	Post Pg
0.13	0.24	(1)	0.09	0.13
0.16	1.02	(2)	0.04	0.43
0.22	0.22	(3)	0.02	0.02
0.32	15.60	(4)	0.40	3.90
0.55	35.86	(5)	0.13	10.60
1.26	2.83	(6)	0.76	1.40
4.32	6.90	(7)	1.50	4.50
5.23	423.50	(8)	0.20	16.60
6.24	100.50	(9)	2.40	58.40
< 0.12	< 0.12	(10)	< 0.02	< 0.02
< 0.12	< 0.12	(11)	< 0.02	< 0.02
< 0.12	< 0.12	(12)	< 0.02	< 0.02

[a] Patients 10, 11 and 12 with undetectable hCG + free ß-hCG and simultaneously free ß-hCG levels (< 0.12 ng/ml and < 0.02 ng/ml, respectively) with no modification after Pg administration, were included separately.

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References

[1] R.O. Hussa, Human chorionic gonadotropin, a clinical marker: review of its biosynthesis, Ligand Rev. 3 (2) (1981) 1–49.

[2] J.C. Fiddes, K. Talmadge, Structure expression and evolution of the genes for the human glycoproteins hormones, Rec. Progr. Horm. Res. 40 (1984) 43–55.

[3] J.G. Pierce, T.F. Parson, Glycoprotein hormones, structure and function, Ann. Rev. Biochem. 50 (1981) 465–495.

[4] H.L. Bradlow, M.K. Schwartz, M. Fleisher, et al., Accumulation of hormones in breast cyst fluid, J. Clin. Endocrinol. Metab. 49 (1979) 778–782.

[5] G. Secreto, C. Recchione, G. Fariselli, E. Grignolio, S. DiPietro, Circulating levels and breast cyst fluid concentrations of human chorionic gonadotropin, progesterone and testosterone in women with gross cystic breast disease, Tumori. 70 (1984) 523–527.

[6] R.B. Greenblatt, V.B. Mahesh, ß-hCG in breast cyst fluids, Ann. N.Y. Acad. Sci. 464 (1986) 632–635.

[7] T.O. Abney, A.Z. Teran, V.B. Mahesh, W.B. Mullins, E.B. Greenblatt, Fibrocystic breast disease: the significance of ß-human chorionic gonadotropin and other polypeptides in breast cyst fluids, Fertil. Steril. 49 (1988) 638–643.

[8] S. Lucchiani, L. Carenza, A. Pala, et al., Evaluation of tumor and trophoblastic marker concentration in breast cyst fluid, Ann. N.Y. Acad. Sci. 586 (1990) 218–229.

[9] P.J. Enriori, C.L. Enriori and Investigators of the Cooperative Group for Breast Cyst Disease Research, Biochemistry of breast cyst fluid. Part II: relationship between androstanodiol glucuronide, dehydroepiandrosterone sulphate, epidermal growth factor and ß-chorionic gonadotropin, Acta Biochim. Clin. Latinoamer. 31 (1995) 395–406.

[10] J. Reforzo–Membrives, C.L. Enriori, M.del C. Cremona, M.del C. Deblauwe, The oral absorption of micronized progesterone, Rev. Argent Endocrinol. Metab. 23 (1986) 11–15.

[11] C.D. Haagensen, C. Bodian, D.E. Haagensen Jr., Gross cystic disease of the breast, in: C.D. Haagensen (Ed.), Breast Carcinoma Risk and Detection, Saunders, Philadelphia, 1981, p. 55.

[12] C.L. Enriori, J.E. Novelli, M.del C. Cremona, R.J.P. Hirsig, P.J. Enriori, Biochemical study of cyst fluid in human breast cystic disease: a review, Breast Cancer Res. Treat. 24 (1992) 1–9.

[13] C.L. Enriori, P.J. Enriori and Investigators of the Cooperative Group for Breast Cyst Disease Research, Biochemistry of breast cyst fluid. Part I: subgroup of breast cysts according to electrolytes, androgen conjugates and epidermal growth factor levels, Breast Dis. 7 (1994) 317–326.

[14] C. Bodian, Benign breast diseases, carcinoma in situ and breast cancer risk, Epidemiol. Rev. 15 (1993) 177–187.

[15] G.D. Braunstein, V. Kamdar, J. Rasor, N. Swaminathan, M.E. Wade, Widespread distribution of a chorionic gonadotropin-like substance in normal human tissues, J. Clin. Endocrinol. Metab. 49 (1979) 917–925.

[16] R. Hoermann, G. Spöttl, R. Moncayo, K. Mann, Evidence for the presence of human chorionic gonadotropin (hCG) and free ß-subunit of hCG in the human pituitary, J. Clin. Endocrinol. Metab. 71 (1990) 179–186.

[17] P. Licht, H. Cao, Z.M. Lei, Ch.V. Rao, W.E. Merz, Novel self-regulation of human chorionic gonadotropin biosynthesis in term pregnancy human placenta, Endocrinology 133 (1993) 3014–3025.

[18] A.J. Rao, K.S.S. Prasad, S.C. Sharma, V.S.R. Subbarayan, Role of 17ß-estradiol and progesterone in the regulation of synthesis and secretion of chorionic gonadotropin by the first trimester human placenta, J. Steroid Biochem. Molec. Biol. 53 (1995) 233–239.

[19] J. Russo, I.H. Russo, Differentiation and breast cancer, Medicina. (Buenos Aires) 57 (2) (1997) 81–91.

[20] I.H. Russo, M. Koszalka, J. Russo, Effect of human chorionic gonadotropin on mammary gland differentiation and carcinogenesis, Carcinogenesis 11 (1990) 1849–1855.

[21] I.H. Russo, J. Russo, Role of hCG and inhibin in breast cancer (review), Int. J. Oncol. 4 (1994) 297–306.

[22] R.A. Badwe, D.Y. Wang, W.M. Gregory, et al., Serum progesterone at the time of surgery and survival in women with premenopausal operable breast cancer, Eur. J. Cancer 30A (1994) 445–448.

[23] S. Juricskay, I. Szabó, K. Kett, Urinary steroids at time of surgery in postmenopausal women with breast cancer, Breast Cancer Res. Treat. 44 (1997) 83–89.