Epidermal Growth Factor in Breast Cyst Fluid: Relationship with Intracystic Cation and Androgen Conjugate Content

Francesco Boccardo, Guglielmo Valenti, Silvia Zanardi, Giannamaria Cerruti, Tiziana Fassio, Paolo Bruzzi, Vincenza De Franchis, Antonina Barreca, Patrizia Del Monte, and Francesco Minuto

Servizio di Oncologia Clinica [F. B., G. V., S. Z., G. C., T. F.] and Servizio di Epidemiologia Clinica [F. B.], Istituto Nazionale per la Ricerca sul Cancro; Laboratorio di Analisi Chimico-Cliniche, Ospedale S. Martino [V. D. F.]; and Cattedra di Fisiopatologia Endocrina, Università di Genova [A. B., P. D. M., F. M.], Viale Benedetto XV 10, 16112, Genova, Italy

ABSTRACT

In recent years, several studies focused on the biochemical analysis of breast cyst fluid composition. It has been shown that breast cysts lined by apocrine epithelium contain higher levels of potassium and dehydroepiandrosterone-sulphate as compared to cysts lined by flattened cells, and that women with apocrine cysts are more likely to develop breast cancer. In the present study, we measured the intracystic levels of sodium (Na+), potassium (K+), dehydroepiandrosterone-sulphate (DHEA-S), and epidermal growth factor (EGF), a factor which could play a role in the autocrine or paracrine control of breast cancer cell growth as recently proposed by some investigators. Breast cyst fluids obtained by fine-needle aspiration from 86 women with gross cystic breast disease were assayed:

On the basis of the relative intracystic concentrations of Na+ and K+ two main classes of cysts were defined. An arbitrary cut-off value of 3 for the Na+/K+ ratio seemed adequate to separate these two types of cysts. An inverse relationship was found between the Na+/K+ ratio and DHEA-S concentration, median levels of the androgen conjugate being 3615 μg/dl in Na+/K+ < 3 cysts and 480 μg/dl in Na+/K+ > 3 cysts (P < 0.001). EGF levels were found to be significantly higher in Na+/K+ < 3 cysts as compared to Na+/K+ > 3 cysts: 103.26 ng/ml versus 57.22 ng/ml, respectively (P < 0.001). EGF appeared inversely correlated with total protein concentration in the Na+/K+ > 3 cysts, while in the Na+/K+ < 3 cysts high EGF levels were observed independently of total protein content. In addition, a direct correlation was found between EGF and DHEA-S concentrations. On the basis of these results, the hypothesis can be made that EGF, which is measurable in all breast fluids tested and is nearly undetectable in plasma, is actually produced by the epithelium lining the cyst wall, particularly as far as the Na+/K+ < 3 cysts are concerned. In view of our results this type of cyst, which has been shown to be lined by apocrine epithelium, appears to be characterized by high DHEA-S and EGF levels. It is suggested that the latter finding could provide a clue for understanding the increased risk of subsequent breast cancer in women bearing apocrine cysts.

INTRODUCTION

Women with GCD of the breast are at increased risk of breast cancer (1-4). It has been suggested that the natural history of this disease is related to prevalent cyst type (5). Several studies have clearly demonstrated that it is possible to identify cyst type by biochemical analysis of breast cyst fluid (6-9). In particular, the intracystic sodium/potassium (Na+/K+) ratio has been suggested as a discriminating factor, cysts lined by apocrine epithelium showing a lower ratio as compared to cysts lined by flattened cells (10). Interestingly, a direct relationship between intracystic K+ and DHEA-S levels was also demonstrated (9, 11).

It has been recently demonstrated that EGF is detectable in breast cyst fluids, although in extremely variable amounts (12, 13). This and other GFs, such as IGF-1 and TGF-α, are at present believed to play a pivotal role in the autocrine and paracrine growth regulation of human breast cancer (14, 15).

No data concerning either the distribution of EGF in different cyst types or, in particular, the relationship between EGF intracystic levels and cation and androgen conjugate breast cyst fluid content, have been reported so far. Our study was designed to answer these specific questions.

MATERIALS AND METHODS

Biochemical Analysis of Breast Cyst Fluids. 95 breast cyst fluid samples have been obtained through fine-needle aspiration from 86 patients: 12 in postmenopause, as defined by absence of menses since at least 12 months and elevated gonadotropin circulating levels, and 74 in premenopause. Patients' median age was 47 years (range, 32-55). In all cases it was possible to assess the intracystic levels of EGF, Na+, and K+. Protein assay was available in 94 samples. In 89 samples it was also possible to determine intracystic DHEA-S levels.

Na+ and K+ were determined by an ion-selecrive electrode technique (Electrolyte 2 Analyzer, Beckman).

Human EGF was measured in untreated cyst fluid by radiimmunoassay using the immunochromes and trace provided by Amersham (UK); the standard curve was performed with a pure human EGF preparation obtained by DNA recombinant technology (Amgen, Thousand Oaks, CA). Sensitivity of this assay was 40 pg/tube. In preliminary experiments breast fluids assayed at different dilutions showed a parallel cross-reaction with the standard. Final dilution of unknown samples was chosen in order to give a concentration ranging between 0.1 and 2.5 ng/tube. Interassay coefficient of variation was 11.5% at a concentration of 1.85 ng/tube.

DHEA-S was measured by radiimmunoassay in untreated samples using a commercially available kit (Immunochrom Chemical Corporation, Carsson, CA). Sensitivity of this assay was 0.6 ng/tube and interassay coefficient of variation was 10% at a concentration of 50 ng/tube.

Proteins were determined by a photometric method, according to Bradford (16).

Statistical Methods. The distribution of DHEA-S and EGF values was heavily skewed. Normalization of the data through logarithmic transformation resulted in a satisfactory stabilization of the variance. Therefore, log-normalized values were used in all statistical analyses. The distribution of protein concentration failed to show any significant deviation from normality and, consequently, absolute values were used in the analyses. Standard linear regression techniques were employed to study the relationships between the different parameters on study.

The Na+/K+ ratio, when log-normalized values were analyzed, showed a bimodal distribution, allowing to distinguish two main classes of cysts. The comparison between protein, EGF, and DHEA-S levels in the two types of cysts was performed by means of the Mann-Whitney U test (two-tailed test) (17). Furthermore, in order to take into account the effect of differing protein concentrations in the two types of cysts, analysis of covariance was carried out using EGF or DHEA-S as the dependent variable and using the two Na+/K+ ratio classes (coded 0 and 1) and protein concentration as covariates. The presence of interaction (effect modification) between the two covariates was assessed as well (18).
RESULTS

Analysis of cation content of breast cyst fluids allowed to distinguish two main populations of cysts, one with a low and the other with a high Na⁺/K⁺ ratio. In fact, the log Na⁺/K⁺ ratio showed a bimodal distribution, and a cut-off point of 3, which appeared to best separate the two populations of cysts, was arbitrarily chosen (Fig. 1). Sixty-five out of 95 breast cyst fluids showed a Na⁺/K⁺ ratio < 3.

DHEA-S levels appeared inversely correlated with the Na⁺/K⁺ ratio, and consequently DHEA-S concentration in the Na⁺/K⁺ < 3 cysts was significantly higher than that found in the cysts with a Na⁺/K⁺ ratio > 3 (Fig. 2 and Table 1). An inverse relationship was also found between DHEA-S and protein concentration, which was significantly lower in the Na⁺/K⁺ < 3 cysts (Table 1). Analysis of covariance showed that, after adjustment for protein values, DHEA-S concentration was still significantly higher in the cysts with a low Na⁺/K⁺ ratio. No evidence of interaction between the two covariates was observed (Table 2).

Intracystic EGF concentration was inversely correlated with the Na⁺/K⁺ ratio, and was significantly higher in the Na⁺/K⁺ < 3 cysts (Fig. 3, Table 1). Again, EGF was inversely and significantly correlated with protein concentration. Analysis of covariance provided somewhat unexpected results (Table 3). Both cyst class and protein concentration appeared significantly correlated with EGF levels. However, when the interaction (effect modification) between cyst class and protein concentration was taken into account, the correlation between each of these two factors and EGF levels was no longer significant, while the significance of the interaction term (P = 0.012) suggested that the relationship between the two types of cysts and EGF levels was dependent on protein concentration. The final model seemed to indicate that cysts with a Na⁺/K⁺ ratio < 3 contain high EGF concentrations (between 100 and 120 ng/ml) independently of protein concentration, while a strong and significant negative correlation between EGF and proteins was observed in cysts with a Na⁺/K⁺ ratio > 3 (Fig. 4). A further attempt to model the interrelationships among Na⁺/K⁺ ratio, EGF levels, and protein concentration using Na⁺/K⁺ ratio as a continuous variable confirmed these results (data not shown). Finally, a strong direct relationship was found between DHEA-S and EGF concentrations (Fig. 5).

No significant differences were observed between pre- and postmenopausal women as far as cyst-type, intracystic EGF and DHEA-S levels are concerned (data not shown).

DISCUSSION

GCD of the breast is a common problem among women in Western countries (1). Basically, from a morphological point of view, breast cysts are classified as flattened or apocrine depending upon the epithelial lining (19).

It has been proposed that the natural history of GCD is strictly related to prevalent cyst type. In fact patients with a single cyst are more likely to have a flattened rather than an apocrine cyst. On the contrary, multiple cysts, whether simultaneous or sequential in any individual patient, are usually found to be all of the same type, and more commonly apocrine than flattened (5).

Dixon et al. (10) have shown the existence of a close relationship between cation content of breast cyst fluid and cyst type. In particular, apocrine cysts were found to contain much higher levels of K⁺ than flattened ones (10). The same investigators suggested that breast cysts can be classified according to their electrolyte content and, based on their data, proposed a cut-off Na⁺/K⁺ value of 3 (9). The results we obtained in the present research are in keeping with those reported by them.

In addition, higher intracystic androgen conjugate levels, especially DHEA-S, were found in the low Na⁺/K⁺ ratio cysts by these and other authors (9, 11). In accordance with the aforementioned data, we also found higher DHEA-S concentrations in this type of cysts.

Although breast cysts do not represent per se a preneoplastic lesion, GCD can be regarded as a premalignant condition. In fact, based on a large number of studies, the development of cysts results in a 2- to 4-fold greater risk of subsequent breast cancer (1–4). An association has been found between apocrine changes, epithelial hyperplasia, and noninvasive carcinoma in breasts removed for invasive breast cancer (19–25), suggesting the existence of a strict linkage between breast cancer risk and a history of apocrine cyst aspiration (25).

There is a mounting evidence that growth factors may be involved in the control of normal and neoplastic cell growth. The mitogenic activity of EGF and IGFI-1 has been demonstrated on normal mammary epithelium and on some human derived breast cancer cell lines (26–31). These cell lines possess specific receptors for IGF-I and EGF, and IGF-1 and EGF receptors have also been described in human breast cancer (14, 31–35). Finally, some of these cell lines, like MCF-7, ZR-75-
**Table 2** DHEA-S concentration (log) as a function of cyst type (Na+/K+ ratio <3 vs. Na+/K+ ratio >3) and protein concentration. Analysis of covariance.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Sum of squares</th>
<th>DF*</th>
<th>F ratio</th>
<th>P</th>
<th>Regression coefficient (SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst type (Na+/K+&lt;3=0; Na+/K+&gt;3=1)</td>
<td>93.70</td>
<td>1</td>
<td>75.02</td>
<td>&lt;0.001</td>
<td>-2.10 (0.263)</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>8.44</td>
<td>1</td>
<td>6.76</td>
<td>0.011</td>
<td>-0.04 (0.016)</td>
</tr>
<tr>
<td>Cyst type × protein concentration</td>
<td>2.33</td>
<td>1</td>
<td>1.89</td>
<td>NS*</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>105.07</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>209.54</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* DF, degrees of freedom.
* Predicted unit change in log-DHEA-S per unit change in the covariate. Constant = 8.63, SE = 0.227. Multiple correlation coefficient = 0.70.

---

Fig. 3. Relationship between log Na+/K+ ratio (X) and log EGF (Y) levels in breast cyst fluids. \( Y = -0.357X + 4.389, r = -0.501, P < 0.001. \)

1, MDA-MB-231, and EVSA-T, were recently shown to produce GFs in vitro, including IGF-I and TFG-α (14, 35, 36), an EGF-related growth factor which interacts with the EGF-receptor. These findings argue in favor of the hypothesis that GFs are involved in the autocrine and paracrine control of breast cancer cell growth.

In our study we have shown that variable amounts of EGF are present in breast cyst fluid. These data confirm and extend previous observations by Jaspar et al. and Collette et al. (12, 13). We have also found a different distribution of this GF according to the cation content. In fact EGF levels were significantly higher in the cysts with a low Na+/K+ ratio than in the high Na+/K+ ratio cysts.

It is a major problem to explain the existence of variable concentrations of EGF in breast cyst fluid. EGF has been found in human milk and colostrum and could be produced by the epithelium lining the cyst wall, as already hypothesized by Collette et al. (13). The finding that plasma EGF concentration is extremely low, with mean values of 0.13–0.16 ng/ml (37), strongly argues in favor of this hypothesis, considering that the mean intracystic level in our series was 111.45 ng/ml.

Our results suggest that the epithelium lining the low N+/K+ ratio cysts may be especially active in EGF production. High EGF levels are detectable in this type of cysts, independently of protein concentration. By contrast, in the high Na+/K+ ratio cysts EGF is present in significantly lower amounts and appears correlated with protein concentration.

Table 3 EGF concentration (log) as a function of cyst type (Na+/K+ ratio <3 versus Na+/K+ ratio >3) and protein concentration. Analysis of covariance.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Sum of squares</th>
<th>DF*</th>
<th>F ratio</th>
<th>P</th>
<th>Regression coefficient (SE)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyst type (Na+/K+&lt;3=0;Na+/K+&gt;3=1)</td>
<td>32.12</td>
<td>1</td>
<td>27.92</td>
<td>&lt;0.001</td>
<td>-0.040 (0.460)</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>11.67</td>
<td>1</td>
<td>10.1</td>
<td>0.002</td>
<td>-0.011 (0.019)</td>
</tr>
<tr>
<td>Cyst type × protein concentration</td>
<td>7.52</td>
<td>1</td>
<td>6.5</td>
<td>0.012</td>
<td>-0.072 (0.028)</td>
</tr>
<tr>
<td>Error</td>
<td>103.52</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>154.84</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* DF, degrees of freedom.
* Predicted unit change in log-EGF per unit change in the covariate. Constant = 4.80, SE = 0.254. Multiple correlation coefficient = 0.58.

At the moment, the inverse relationship existing between EGF and proteins in the high Na+/K+ ratio cysts does not find a clear explanation, and deserves further investigations. The finding of high EGF levels in the low Na+/K+ ratio cysts is novel and as these cysts have been reported to be lined by apocrine epithelium (10), is consistent with the direct relationship previously found between intracystic EGF levels and the levels of gross cystic disease fluid protein-15 (13). This breast cyst fluid protein has been suggested as a biochemical expression of apocrine secretion due to its high concentration in hormone-dependent apocrine glands such as axillary and anogenital sweat glands (13). The presence of high EGF levels in low Na+/K+ ratio cysts, namely apocrine cysts, might provide a key to understanding the mechanisms which determine the high risk for breast cancer associated with these cysts.
The direct relationship we found between intracycstic EGF and DHEA-S levels is also worth noting. High EGF levels have been found in human submaxillary salivary glands: this gland in some other mammalian species produces pheromones under androgen modulation through acocrine modalities (38, 39). It has also been reported that testosterone administration increases EGF concentration in the mouse submaxillary gland (40, 41). Accordingly, if we assume that EGF is produced by the acocrine epithelium lining the low Na⁺/K⁺ ratio cysts the strong relationship between this growth factors and DHEA-S levels may suggest that EGF production is androgen modulated also at this level. Of course, the hypothesis that both EGF and DHEA-S are elevated in response to some other common stimulus cannot be ruled out.

Our findings seem to represent a further step towards a better understanding of GCD physiopathology and its relationships with malignant breast pathology.

REFERENCES

Epidermal Growth Factor in Breast Cyst Fluid: Relationship with Intracystic Cation and Androgen Conjugate Content

Francesco Boccardo, Guglielmo Valenti, Silvia Zanardi, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/20/5860

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.