Estrogen Production as a Tumor Marker in Patients with Gonadotropin-producing Neoplasms

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ABSTRACT

Urinary estradiol production rates, plasma estradiol, and peripheral conversion of dehydroepiandrosterone sulfate (DHEAS) to estradiol were explored in patients whose neoplasms made human chorionic gonadotropin and/or human chorionic gonadotropin β fragments. In five men with choriocarcinoma, estradiol production was elevated, plasma estradiol was high, and DHEAS conversion to estradiol ranged from 0.5 to 11.7%. In six patients with "ectopic" gonadotropin-producing tumors, originating from lung, stomach, and liver, estradiol production was elevated to a lesser degree.

Sera from 154 of 864 patients (16.8%) with a wide variety of advanced neoplastic disorders were noted to have minimal elevations of human chorionic gonadotropin β subunit in their sera but had normal luteinizing hormone assays. Plasma estradiol levels in these patients were not elevated, and in only one of 10 patients studied was there slight elevation of the estradiol production rate. Similarly, in only one of ten patients was there measurable conversion of DHEAS to estradiol.

We conclude that, in patients whose neoplastic disorders are associated with major elevations of human chorionic gonadotropin, there is concordant extragonadal production of estradiol via DHEAS, presumably representing an associated trophoblastic function. However, estrogen production is not a sensitive tumor marker in those patients whose neoplasms are associated with minimal elevations of human chorionic gonadotropin β fragments only.

INTRODUCTION

We have previously found increased estrogen production rates in men with trophoblastic neoplasms of testicular and mediastinal origin (12). Urinary estradiol production rates and plasma estradiol levels correlated well with tumor burden and HCG3 production. The increased estrogens appeared to arise from tumor metabolism of circulating DHEAS to estradiol, a biochemical function characteristic of normal placental tissue (1, 2, 9, 13). Since trophoblastic neoplasms appear to produce a variety of hormones and proteins characteristic of normal placental tissue (17, 21, 23), we were interested in exploring whether tumors of nontrophoblastic origin found to ectopically produce HCG and/or its β-subunit (5, 22) are similarly capable of performing other biochemical functions of normal placental tissue.

In the current study, we examined estrogen production rates and estradiol blood levels in patients with ectopic HCG-producing neoplasms and in cancer patients whose sera contained minimal amounts of HCGβ subunit. Estrogen production via the "DHEAS route" was elevated in those patients whose tumors elaborated larger quantities of HCG but was not present in those patients making minimally detectable levels of HCG or its β-subunit. Estradiol production was a less sensitive tumor marker than was measurement of HCG fragments.

MATERIALS AND METHODS

HCG and/or its β-subunit were measured in serum after the method of Vaitukaitis et al. (21), using SB-6 antisera, but without chromatographic separation of the β-subunit from the complete HCG molecule. In this assay, a value of 5 mIU/ml of serum could be differentiated from the blank and was considered positive. Luteinizing hormone (cross-reacting with HCGβ) was measured by radioimmunoassay using a standard kit from Serono Laboratories, Boston, Mass.

Plasma estradiol was measured by radioimmunoassay using the specific antibody of Loriaux. Ether extracts of serum were purified by Sephadex LH-20, prior to assay, as described previously (18).

Urinary estradiol production rates were measured by reverse isotope dilution, following i.v. administration of 3 μCi of [14C]estradiol and urine collection over the next 4 days (12, 18). A one-tenth aliquot was incubated with Helix pomatia enzyme, extracted after the method of Brown (7), and, following chromatographic cleanup, was recrystallized to constant specific activity following addition of pure crystalline estradiol, as described previously (12, 13, 18). Prior to the crystallization procedure, a small aliquot of the purified extract was taken for mass estimate by radioimmunoassay to determine the specific activity of the estrogen for the denominator of the final equation:

\[
\frac{\text{cpm [14C]estriadiol given}}{\text{cpm [14C]per mass of estradiol in purified fraction} \times \text{days}}
\]

Conversion of DHEAS to estradiol (\(^{(3)}\text{H}D\text{HEAS} \rightarrow \text{estradiol}\)) was determined by simultaneous administration of 40 μCi of [3H]DHEAS to the patient along with [14C]estradiol (above) and subsequent purification via "cocrystallization" of the estrogens isolated from the 4-day urine pool. Agreement of 8% in specific activity of 2 successive crystallizations and mother liquors was taken as proof of purification that the [3H]DHEAS had indeed been metabolized to estradiol. In all cases, 5 crystallizations were performed using combinations of ethanol:water and methanol:water, although radiohomogeneity was generally achieved by the third or fourth crystallization.

Five men with trophoblastic tumors arising from testes or...
Gonadotropin Production. In 5 men with histologically proven trophoblastic disease arising from testes or mediastinum, gonadotropin measurements in sera and/or urine using elevated, ranging from 150 mlU/ml (serum) to 107 mlU/day in standard luteinizing hormone antibody were found to be clearly proven trophoblastic disease arising from testes or mediastinum. The clinical and biochemical features of these patients are shown in Table 1. In each case, examination of the primary lesion failed to reveal trophoblastic tissue.

Sera from 864 patients with a variety of nontrophoblastic tumors being followed by our Oncology Service were screened for the presence of HCGβ fragment. Sera found to be positive in a particular patient were taken for estradiol assay as a screening test prior to formal determination of urinary estradiol production rate. In 10 consenting patients whose sera showed detectable levels of HCGβ and in 6 patients whose sera gave several negative readings for HCGβ, formal urinary estradiol production rates and DHEAS conversion to estradiol were determined following i.v. administration of purified radiotracers, as described above. All cancer patients were studied either before or in between courses of chemotherapy. Four patients of comparable age hospitalized for non-cancer-related illnesses were similarly studied as controls.

### RESULTS

#### Gonadotropin Production.

In 5 men with histologically proven trophoblastic disease arising from testes or mediastinum, gonadotropin measurements in sera and/or urine using standard luteinizing hormone antibody were found to be clearly elevated, ranging from 150 mlU/ml (serum) to 10⁷ mlU/day in urine. Using specific HCG antibody, values ranged from 790 mlU/ml to 10⁶ mlU/day. As reported previously the magnitude of HCG production in these patients appeared to correlate with extent of disease.

In 6 patients with ectopic gonadotropin-producing neoplasms of lung, stomach, and liver, the gonadotropin titers were somewhat lower than in patients with true choriocarcinoma, ranging from slightly above normal to excretion values of 10⁶ mlU/day. In the case of patients with choriocarcinoma, HCG measured by specific antibody correlated with the total gonadotropin elevations.

Sera from 864 patients with a variety of neoplastic disorders were screened for the presence of HCG or its β-subunit using specific antisera. In this group, 145 sera (16.8%) gave a positive reading, usually ranging from 5 to 50 mlU/ml as noted in Table 2. By contrast, only 4 of 87 patients hospitalized for non-cancer-related illnesses had sera positive for HCG (4.6%). Of possible interest was the finding that sera from 3 of 5 patients with malignant melanoma were positive for HCGβ, as noted by previous workers (5).

Although less specific assays for luteinizing hormone were not routinely performed in this group of patients, none of the 130 patients tested had elevated luteinizing hormone values.

Serial measurements of HCG were performed on sera from 173 patients. In this group, 102 of 173 patients had sera which on 2 to 8 samplings were persistently negative. Of the 71 patients whose sera were positive for HCGγ, only 11 had persistently positive values on multiple samplings. Presence of this marker proved to be evanescent in 60 of 71 patients, being found only (on average) 42% of the time without apparent correlation to therapy or progress of disease. By contrast, HCGβ was consistently elevated in sera of patients with choriocarcinoma and ectopic HCG-producing tumors, serving as a more reliable tumor marker in these situations.

### Table 1

<table>
<thead>
<tr>
<th>Primary site</th>
<th>Cell type</th>
<th>Stage of disease</th>
<th>Gyneco-mastia</th>
<th>Luteinizing hormone (mlU/day)</th>
<th>HCG (mlU/day)</th>
<th>DSO → estradiol (%)</th>
<th>Urinary estradiol production rate</th>
<th>Estradiol (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. B.*</td>
<td>Mediastinum</td>
<td>Choriocarcinoma</td>
<td>Early; no metastases</td>
<td>No 1.5 x 10⁵</td>
<td>7.9 x 10⁴</td>
<td>0.5</td>
<td>62</td>
<td>13.5</td>
</tr>
<tr>
<td>L. W.*</td>
<td>Testes</td>
<td>Choriocarcinoma</td>
<td>Widespread</td>
<td>Yes 1 x 10⁷</td>
<td>1.1 x 10⁶</td>
<td>11.7</td>
<td>293</td>
<td>40</td>
</tr>
<tr>
<td>J. K.*</td>
<td>Mediastinum</td>
<td>Choriocarcinoma</td>
<td>Widespread</td>
<td>Yes 1 x 10⁷</td>
<td>6.9 x 10⁶</td>
<td>11.0</td>
<td>460</td>
<td>31</td>
</tr>
<tr>
<td>J. L.*</td>
<td>Mediastinum</td>
<td>Choriocarcinoma</td>
<td>Widespread</td>
<td>Yes 1 x 10⁷</td>
<td>5.4 x 10⁶</td>
<td>10.0</td>
<td>605</td>
<td>58</td>
</tr>
<tr>
<td>W. M. C.</td>
<td>Testes</td>
<td>Choriocarcinoma</td>
<td>Widespread</td>
<td>No 8 x 10⁶</td>
<td>5.4 x 10⁶</td>
<td>10.0</td>
<td>910</td>
<td>25</td>
</tr>
</tbody>
</table>

* Values previously reported (12).

### Table 2

<table>
<thead>
<tr>
<th>Origin</th>
<th>HCGβ-positive/total examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>38/218 (17)</td>
</tr>
<tr>
<td>Uterus</td>
<td>8/33 (24)</td>
</tr>
<tr>
<td>Ovary</td>
<td>11/38 (28)</td>
</tr>
<tr>
<td>Prostate</td>
<td>7/35 (20)</td>
</tr>
<tr>
<td>Testes</td>
<td>2/11 (11)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>34/220 (15)</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>7/46 (15)</td>
</tr>
<tr>
<td>Lung</td>
<td>20/101 (20)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>4/87 (5)</td>
</tr>
<tr>
<td>Others</td>
<td>11/69 (16)</td>
</tr>
</tbody>
</table>

Sera analyzed for HCGβ markers

| Non-cancer patients | 4/87 (4.6) |

# Numbers in parentheses, percentages.
Plasma Estradiol. Values for plasma estradiol in the various groups of patients (choriocarcinoma, ectopic trophoblast-like disease, tumors with HCG fragments only, cancer patients without HCG, and noncancerous controls) are noted in Chart 1. In patients with choriocarcinoma, plasma estradiol was significantly elevated, ranging from 135 to 480 pg/ml, and appeared to correlate with urinary estradiol production rates (see below). By contrast, in those patients with ectopic HCG-producing tumors and high production of gonadotropins, estradiol levels were not elevated except in one patient. Other cancer patients with only minor HCG elevations and other groups showed normal plasma estradiol concentrations.

Urinary Estradiol Production Rates. In patients with choriocarcinoma, urinary estradiol production rates were elevated in proportion to the tumor burden, ranging from 62 to 910 µg/day, as noted in Chart 2. In all 6 patients with ectopic gonadotropin producing tumors, the urinary estradiol production rate was also elevated but to a lesser extent, ranging from 97 to 328 µg/day. In contrast to these 2 groups, in patients whose tumors made only small amounts of HCG, urinary estradiol production rate was only minimally elevated in one of the 10 patients (66 µg/day), but values were within the normal range in the other 9 patients studied. In cancer patients with no detectable HCG as well as in noncancerous control patients, the urinary estradiol production rates were normal.

Peripheral Conversion of DHEAS to Estradiol. Extragonadal metabolism of DHEAS to estradiol is plotted for the various groups of patients studied in Chart 3. In all patients with choriocarcinoma, notably 4 of 5, conversion of DHEAS to estradiol was very high at 10 to 11%. In the 6 men with ectopic HCG-producing tumors, all had clearly measurable peripheral metabolism of DHEAS to estradiol, ranging from 0.4 to 3.3%. Assuming normal DHEAS production in these patients, this degree of peripheral conversion to estradiol would account for the increased estradiol production in each patient with choriocarcinoma or ectopic HCG-producing tumors. By contrast, in only 1 of 10 cancer patients with HCG-positive sera was peripheral conversion of DHEAS to estradiol increased to 1.8%. In all others, this value was 0.4% or less, representing the limit of the method. Similarly, in cancer patients without HCG fragments in their sera and in noncancerous control patients, no detectable conversion of DHEAS to estradiol could be measured.

**DISCUSSION**

We have previously shown that, in men with choriocarcinoma of testicular and mediastinal origin, excessive amounts of estradiol were made via peripheral metabolism of DHEAS to estradiol (12). Incubation studies of primary tumor or metastatic tissues obtained at biopsy or postmortem examination showed excessive conversion of substrate DHEAS to estradiol, making it likely that tumor sites were responsible for the extragonadal formation of estradiol in these men. This result was not surpris-
ing, since normal trophoblastic tissue incubated in vitro or in tissue culture (14) or placental perfusion studies (1, 2) have readily shown that trophoblastic tissues are capable of aromatizing C₁₉ substrates, notably DHEAS. In the current study, we extend earlier observations that patients with neoplastic disorders characterized by ectopic production of HCG also make excessive amounts of estradiol via extraglandular metabolism of DHEAS, as noted in patients with choriocarcinoma. It appears, however, that the efficiency of this extraglandular estradiol production is not as great as in choriocarcinoma, since the urinary estradiol production rates were not as elevated, nor was the extent of DHEAS conversion to estradiol. These data in 6 patients with ectopic HCG-producing tumors nonetheless parallel observation of Braunstein, Weintraub and others (5, 10, 11, 17, 23, 24) suggesting that ectopic HCG-producing tumors produce somatomammotropin, HCG fragments, and estrogens, thus retaining many of the biochemical functions of true trophoblastic tissue despite histological dissimilarities (17). In this regard, Rosen’s group reported discordance in production of various protein markers and/or hormones by clones of trophoblast-like tumor cells grown in culture (6, 16), in nude mice (15), and in tumors in clinical studies (4, 19). This group has reported instances of dysynchrony in proportions of gonadotropin and its subunits, somatomammotropin, placental alkaline phosphatase, and other markers under varying conditions. These findings are similar to earlier reports by Kohler et al. (14), who observed differing proportions of various hormones in clones of trophoblastic cells grown in tissue culture. Discordance of various proteins formed by these tumors does not negate the likelihood that these tumors represent a peculiar form of neoplastic retrodifferentiation versus tumors which simply produce repressed proteins.

Earlier studies of Braunstein et al. (5) and Vaitukaitis et al. (22) demonstrated measurable amounts of HCG$_{α}$ fragments in sera of 12% of patients with a variety of neoplastic disorders. This observation has subsequently been confirmed by others (10, 11) including the current study (Table 2). In more recent studies using concentrating techniques, several groups have demonstrated HCG-reacting substances in sera from many cancer patients and in normal tissue extracts from patients free of neoplastic disorders (4, 8). Indeed, Yoshimoto et al. (26) have suggested that an asialo form of the β-subunit of HCG may be made by all normal tissues and that tumor tissues may express the ability to glycosylate this molecule, slowing down its clearance, thus leading to its detection in the sera and tissues.

In the current study, we attempted to relate plasma estradiol, urinary estradiol production rate, and extragonadal metabolism of DHEAS to estradiol as a possible concordant tumor marker with HCG$_{α}$. Our studies show that estradiol production and blood levels are not sensitive markers of trophoblast-like cancers. In those patients whose neoplastic disorders were associated with major elevations of HCG, we could indeed demonstrate increased estradiol production rates and increased conversion of DHEAS to estradiol. However, in those cancer patients whose sera merely registered a positive in HCG$_{α}$ testing, estradiol is not elevated and is thus not a concordant-sensitive tumor marker.

REFERENCES

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