Estrone to Estradiol Conversion by Blood Mononuclear Cells in Normal Subjects and in Patients with Mammary and Nonmammary Carcinomas

A. Borkowski, M. Dosogne, P. Declercq, C. Muquardt, and D. Machin

Service de Médecine Interne et Laboratoire d’Investigation Clinique, H. J. Tagnon, Institut Jules Bordet, Tumour Centre of the Free University of Brussels [A. B., M. D., P. D., C. M.], and the Data Center of the E.O.R.T.C., Brussels 1000, Belgium [D. M.]

ABSTRACT

The conversion rate of estrone to 17ß-estradiol was investigated in a 3-day culture of human blood mononuclear cells. Per 10 million cells the mean percentage of conversion was 5.64 (S.E., 0.24) in 56 normal subjects, and there was no significant difference among men, premenopausal women, and postmenopausal women. The conversion was not modified by the addition of steroid sex hormones in the medium or by an increase of the glucose concentrations.

It was significantly lower (p < 0.001) in 22 patients with a localized mammary carcinoma (mean, 3.77; S.E., 0.26) and in 16 patients with localized nonmammary carcinomas (mean, 4.28; S.E., 0.39) but returned progressively to normal or high values when the tumors became generalized.

In the patients with mammary carcinomas, it was significantly lower (p < 0.025) than in those with nonmammary carcinomas but was still, as in normal subjects, definitely higher than the reverse (17ß-estradiol to estrone) reaction.

The low estrone to 17ß-estradiol conversion rate appeared to be present at the very beginning of the disease; its increase with the generalization of the tumor might be attributable to an activation of the monocytes because a positive correlation (r = 0.554; p ~ 0.02) between the number of monocytes and the conversion rate was then detected; this increase might also be a useful and early index of the spread of the cancer.

INTRODUCTION

The immune system appears to play a critical role in the host defenses against a malignant neoplasm (7, 10, 13). Varying degrees of lymphocytic infiltration have been shown in primary and metastatic cancer (11). This immune response includes a direct killing of tumor cells by sensitized T-lymphocytes, monocytes, or macrophages and an antibody-dependent cell-mediated cytotoxicity (3-6, 22). The mechanism by which the blood mononuclear cells kill the tumor cells is unclear but most probably involves the release of some toxic factors that disrupt the cell membrane or interfere with DNA function (18). On the other hand, the growth of some frequent malignant tumors, like breast carcinoma, is also under endocrine control, the role of the steroid sex hormones and particularly of the estrogens being prominent in this regard (12). A question arises therefore. Could the blood mononuclear cells control the growth of the hormone-dependent tumors and eventually kill the malignant cells by an endocrine mechanism?

This problem was approached by investigating the conversion of estrone to 17ß-estradiol in a 3-day culture of human blood mononuclear cells; mononuclear cells from women with localized or generalized mammary carcinomas were compared to those from normal subjects and from patients with other malignant tumors. Indeed, the peripheral tissues are capable of producing steroid sex hormones from precursor steroids in blood (2, 8, 9, 21); the conversion of estrone to 17ß-estradiol is particularly interesting in this regard since it regulates the biological activity of circulating estrone in the premenopausal women (9) and is the main source of biologically active estrogens in the postmenopausal women (6, 21); this conversion is catalyzed by a widespread enzyme, 17ß-estradiol dehydrogenase (19, 20).

MATERIALS AND METHODS

Subjects. The normal subjects were 56 healthy volunteers ranging in age from 21 to 82 years: 20 men and 20 premenopausal women (mean age, 27 years; S.E., 0.8), 16 postmenopausal women (mean age, 59 years; S.E., 2.3).

The patients with mammary carcinoma included 22 patients with a localized tumor (7 pre- and 15 postmenopausal women; mean age, 61 years; S.E., 3.0) and 16 patients with a generalized tumor (2 pre- and 14 postmenopausal women; mean age, 64 years; S.E., 2.8). In most of the patients with a localized tumor, the axillary lymph nodes were subsequently explored by the surgeon and analyzed by the pathologist.

The patients with nonmammary carcinomas served as cancerous controls (Table 1). Sixteen had a localized tumor (7 men, 3 pre- and 6 postmenopausal women; mean age, 61 years; S.E., 3.8) and 18 had a generalized tumor (12 men and 6 postmenopausal women; mean age, 64 years; S.E., 2.1). The localized tumors were subsequently operated on and thus also explored by the surgeon.

All the patients were carefully evaluated for metastases; the standard work-up in breast carcinoma included a chest X-ray, a scan of skeleton, blood liver tests, and a scan of the liver; if 1 of the latter 2 was abnormal, a laparoscopy and a liver biopsy were performed. Patients with a viral or a bacterial infection, patients who had been treated with hormones during the past 12 months, and patients who had ever been treated for cancer by surgery, chemotherapy, or radiotherapy were excluded from the investigation.

1 Supported by the Fondation Cancérologique de la Caisse Générale d’Epargne et de Retraite de Belgique, by the Fonds National de la Recherche Scientifique, and by the Ministère Belge de la Politique Scientifique within the framework of the Association Euratom-University of Brussels-University of Pisa.

2 To whom requests for reprints should be addressed.

Received December 6, 1977; accepted April 10, 1978.
Isolation of Blood Mononuclear Cells and Culture. Freshly drawn blood was heparinized with Calparine (Choay). The blood samples were obtained in the laboratory on a blind basis. Lymphocyte-rich preparations were isolated by a sodium metrizoate:Ficoll density gradient. Leukocyte counts (modified Neubauer hemocytometer) were based on a count of at least 1000 cells. For differential counts (500 cells, 3 slides) samples on pulled coverslips were fixed in 99% methanol and stained with Giemsa. The leukocytes were suspended in 5 ml of Eagle's minimal essential medium 138 (Grand Island Biological Co., Grand Island, N. Y.) (14) supplemented with 20% fetal calf serum (inactivated during 1 hr at 56°), 2% glutamine, 1% phytohemagglutinin, and 1% pokeweed mitogen in the presence of streptomycin and penicillin. The initial cell density was \( \approx 1 \times 10^{10} / \text{ml} \). After addition of approximately \( 1.6 \times 10^8 \) dpm [\( 2,4,6,7^{-3} \text{H}\)]estrone per ml (specific activity, 90 Ci/mmol), the cultures were incubated during 72 hr at 37° in a controlled atmosphere at 5% \( \text{CO}_2 \), in humidified air. The incubations were terminated with the addition of inert \( 17\beta \)-estradiol and approximately 25,000 dpm \( 17\beta^{-[4-14\text{C}]\text{estradiol}} \) (specific activity, 55 mCi/mmol).

All the markers were obtained from Radiochemical Centre, Amersham (England) and purified by Sephadex chromatography before use (16).

Each experiment was run in duplicate and with blanks (i.e., medium plus radioactive steroids but no leukocytes). Monocyte contamination averaged 14% (range, 1 to 26; S.E., 1.4) in the normal subjects, 13% (range, 2 to 28, S.E., 2.0) in localized mammary carcinomas, 12% (range, 1 to 31; S.E., 3.0) in generalized mammary carcinomas, 17% (range, 6 to 33; S.E., 1.8) in localized nonmammary carcinomas, and 22% (range, 0 to 33; S.E., 1.9) in generalized nonmammary carcinomas. Only the latter percentage was significantly higher than in the normal controls (\( p < 0.01 \)).

Measurement of Estrone to \( 17\beta \)-Estradiol percentage of conversion. The whole culture was extracted 3 times with 20 ml of ether after it had been shown in preliminary experiments that there was practically no storage of estradiol within the cells. The extract was washed with water, evaporated, and chromatographed twice on Sephadex LH-20 columns (16) with methylene chloride:methanol (95:5, v/v). \( 17\beta \)-Estradiol was then acetylated in 40 \( \mu l \) of benzene:acetic anhydride (5:1, v/v) and 30 \( \mu l \) of pyridine at 37° for 24 hr. The diacetate was extracted with methylene chloride and purified by thin-layer chromatography (plates of Silica Gel 60 F-254) successively in benzene:ethyl acetate (8:2, v/v) and methylene chloride:ether (97:3, v/v). After addition of cold carrier, the \( 17\beta \)-estradiol diacetate was crystallized 3 times in hexane:acetone until constancy of the \( \text{H:}^{14\text{C}} \) ratio in crystals and mother liquors. The \( \text{H:}^{14\text{C}} \) ratio was measured on aliquots at each step of the purification. The latter was practically achieved after the second thin-layer chromatography. The estrone to \( 17\beta \)-estradiol percentage of conversion was obtained by comparing the \( \text{H:}^{14\text{C}} \) ratios after culture extraction and after the last crystallization; it was expressed as the percentage of conversion per 10 million cells.

In a few experiments the estrone to \( 17\beta \)-estradiol percentage of conversion was compared with the \( 17\beta \)-estradiol to estrone percentage of conversion. \([\text{H}]\)Estrone produced from \([\text{H}]\)estradiol in separate cultures was then isolated through the same purification steps after the addition of \([^{14}\text{C}]\)estrone for the correction of losses.

Statistical Analysis. The estrone to \( 17\beta \)-estradiol conversion rates of the various subgroups of patients were compared by means of the nonorthogonal analysis of variance technique (1) since the numbers of observations in each subgroup were not equal. The subsequent group comparisons were made with the variance ratio test. Such a test requires the assumption of normality of the data within each subgroup. Checks on this suggested that all the data could be assumed to be normally distributed if the logarithms of the data were taken before analysis. Analyses were first carried out after logarithmic transformation and then repeated on the raw (untransformed) data. The 2 approaches led to identical conclusions, however, so that the results are presented in terms of the actual percentage of conversion for ease of interpretation.

The percentages of monocytes were compared in the same way.

RESULTS

Estrone to \( 17\beta \)-Estradiol Percentage of Conversion According to the Number of Mononuclear Cells (Chart 1). As shown in Chart 1, the percentage of conversion was proportional to the number of blood mononuclear cells; on the other hand, the conversion rates obtained in the absence of cells (blanks) have always been negligible. The blood mononuclear cells were thus responsible for total \( 17\beta \)-estradiol production, and all the data could be expressed...
as a percentage of conversion per $10 \times 10^6$ cells.

Estrone to $17\beta$-Estradiol Percentage of Conversion According to the Duration of the Incubation (Chart 2). The percentage of conversion increased regularly with time during the 3 days of culture, and the efficiency of the blood mononuclear cells in converting estrone to $17\beta$-estradiol was thus fairly constant throughout our 3-day experiments.

Estrone to $17\beta$-Estradiol Conversion Rate According to the Concentration of the Substrate (Chart 3). The absolute conversion of estrone to $17\beta$-estradiol was proportional to the concentration of estrone in the medium within a range of 0 to 2,500 pg/ml; within this range, which included our usual experimental conditions (approximately 2,000 pg estrone/ml), $17\beta$-estradiol dehydrogenase was thus presumably working much below V$_{max}$ and the estrone to $17\beta$-estradiol percentage of conversion was practically independent of the concentrations of free estrone in the medium.

Estrone to $17\beta$-Estradiol Percentage of Conversion in Normal Subjects (Chart 4, Table 2). The mean percentages of conversion in the 20 men, 20 premenopausal women, and 16 postmenopausal women were respectively: 5.19 (S.E., 0.34); 5.53 (S.E., 0.38); and 6.34 (S.E., 0.52). There was no significant difference between the 3 populations, and the general mean percentage of conversion was: 5.64 (S.E., 0.24).

It is of interest that under the same experimental conditions the conversion of estrone to $17\beta$-estradiol by the corresponding RBC (this was tested in 6 normal subjects) was relatively negligible (below 0.3%/10 $\times 10^6$ cells).

Estrone to $17\beta$-Estradiol Percentage of Conversion in Patients with Mammary Carcinomas (Chart 5, Table 2). The percentage of conversion in the 22 patients with a localized mammary carcinoma (mean, 3.77; S.E., 0.26) was significantly lower ($p = 0.001$) than in the 16 patients with a generalized mammary carcinoma (mean, 5.37; S.E., 0.31); it was also very significantly lower than in the normal subjects ($p < 0.001$).

Actually, it appears that the more localized the tumor was, the lower was the estrone to $17\beta$-estradiol percentage of conversion; in the patients with a localized tumor and negative axillary lymph nodes, the mean percentage of conversion was 3.39 (S.E., 0.26, $N = 9$), whereas with positive axillary lymph nodes the mean was 4.13 (S.E., 0.55; $N = 7$).

Estrone to $17\beta$-Estradiol Percentage of Conversion in Patients with Nonmammary Carcinomas (Chart 5; Table 2). The percentage of conversion in the 16 patients with a localized carcinoma (mean, 4.28; S.E., 0.39) was again very significantly lower ($p < 0.001$) than in the 18 patients with a generalized carcinoma (mean, 6.54; S.E., 0.58); it was also significantly lower ($p = 0.001$) than in the normal subjects.

Among the patients with a localized tumor, the lowest conversion rates, i.e., below 3%, were found, respectively, in 2 patients with a small cancerized polyp (of the bladder and of the rectum, subsequently treated by simple endoscopic resection) and in 2 patients with a small p.o. carcinoma (of the tongue and of the gum).

Mammary Carcinomas versus Nonmammary Carcinomas (Chart 5; Table 2); Correlation between the Number of Monocytes and the Estrone to $17\beta$-Estradiol Conversion Rate. The estrone to $17\beta$-estradiol conversion rates were significantly lower in the patients with mammary carcinomas than in those with nonmammary carcinomas ($p < 0.025$).

The correlation between the estrone to $17\beta$-estradiol percentage of conversion and the number of monocytes in the culture was investigated in each subgroup of normal subjects and patients; a significant correlation was found only in the patients with nonmammary generalized carcinomas, i.e., those with the highest number of monocytes and the highest estrone to $17\beta$-estradiol conversion rate ($r$, 0.554; $p = 0.02$).

Estrone to $17\beta$-Estradiol versus $17\beta$-Estradiol to Estrone Percentage of Conversion (Table 3). This was investigated in 5 normal subjects and 5 patients with mammary carcinomas by a paired 2-sided t test. The estrone to $17\beta$-estradiol conversion was always and definitely higher than the reverse conversion ($p < 0.001$).
Table 2
Estrone to 17β-estradiol percentage of conversion by the blood mononuclear cells

<table>
<thead>
<tr>
<th></th>
<th>Mammary carcinomas</th>
<th>Nonmammary carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>(N² = 56)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.64 ± 0.24</td>
<td>6.54 ± 0.58</td>
</tr>
<tr>
<td>Localized</td>
<td>3.77 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>(N = 22)</td>
<td>4.28 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td>5.37 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>(N = 16)</td>
<td>4.28 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Localized</td>
<td>6.54 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>(N = 18)</td>
<td>4.28 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Axillary lymph nodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3.39 ± 0.26</td>
<td>4.13 ± 0.55</td>
</tr>
<tr>
<td>(N = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

α N, number of individuals.

Table 4
Influence of glucose and steroid sex hormones in the medium

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal womanα</th>
<th>Postmenopausal womanβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (glucose, 100 mg/100 ml)</td>
<td>4.67</td>
<td>5.08</td>
</tr>
<tr>
<td>Glucose (100 mg/100 ml): insulin (20 μunits/ml)</td>
<td>4.84</td>
<td>5.32</td>
</tr>
<tr>
<td>Glucose (200 mg/100 ml): insulin (200 μunits/ml)</td>
<td>4.75</td>
<td>4.49</td>
</tr>
<tr>
<td>Glucose (300 mg/100 ml): insulin (200 μunits/ml)</td>
<td>4.69</td>
<td>4.16</td>
</tr>
<tr>
<td>Glucose (400 mg/100 ml): insulin (200 μunits/ml)</td>
<td>4.88</td>
<td>4.49</td>
</tr>
<tr>
<td>Diethylstilbestrol 700 pg/ml</td>
<td>4.84</td>
<td>4.81</td>
</tr>
<tr>
<td>70,000 pg/ml</td>
<td>4.97</td>
<td>4.64</td>
</tr>
<tr>
<td>17β-Estradiol 700 pg/ml</td>
<td>4.70</td>
<td>3.86</td>
</tr>
<tr>
<td>7,000 pg/ml</td>
<td>4.97</td>
<td>4.25</td>
</tr>
<tr>
<td>Testosterone 6 ng/ml</td>
<td>5.20</td>
<td>3.84</td>
</tr>
<tr>
<td>600 ng/ml</td>
<td>4.84</td>
<td>4.81</td>
</tr>
<tr>
<td>Progesterone 10 ng/ml</td>
<td>4.88</td>
<td>5.10</td>
</tr>
<tr>
<td>1000 ng/ml</td>
<td>4.86</td>
<td>5.07</td>
</tr>
<tr>
<td>Androstenedione 1 ng/ml</td>
<td>5.07</td>
<td>4.91</td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>4.70</td>
<td>4.82</td>
</tr>
<tr>
<td>Dehydroepiandrosterone 4 mg/ml</td>
<td>4.93</td>
<td>4.40</td>
</tr>
<tr>
<td>400 mg/ml</td>
<td>5.07</td>
<td>4.60</td>
</tr>
</tbody>
</table>

α Age, 30 years; percentage of monocytes, 18.
β Age, 29 years; percentage of monocytes, 12.
γ Age, 58 years; percentage of monocytes, 6.
δ Same results without insulin.

Influence of Glucose and Hormone Concentrations in the Medium (Table 4). This was investigated on the blood mononuclear cells from 3 normal subjects. As shown in Table 4, the estrone to 17β-estradiol conversion rate was not significantly modified by any steroid sex hormone or precursor; it was not modified either when the concentration of glucose increased up to 4 times in the medium.

DISCUSSION
The conversion of estrone to estradiol in humans as measured in blood is of the order of 5% (2); however, this conversion rate must be very different in the various tissues depending on local uptake, local enzyme, coenzyme, and hydrogen ion concentrations (15, 19). In our experimental system, under conditions comparable to those prevailing in
vivo, the human blood mononuclear cells converted estrone to 17β-estradiol efficiently. Everything being equal this conversion was considerably higher than by the corresponding RBC. It was a first-order reaction from very low up to our experimental estrone concentrations; 17β-estradiol dehydrogenase was thus presumably working much below its maximum rate; this also suggests that our observations were relevant to physiological free estrone concentrations. The reduction of the 17-ketone was favored, whereas the overall direction of this oxidation-reduction enzymatic system, as measured in peripheral blood, favors the oxidative pathway, i.e., the inactivation of 17β-estradiol to estrone (2). Unlike what happens in target tissues such as the endometrium (9), estradiol formed by the mononuclear cells was rapidly released into the medium. Consequently, with the limitations of in vitro to in vivo extrapolations, it appears that the blood mononuclear cells might be a significant source of estradiol in humans; since they circulate throughout the body, cross the capillary bed, and can selectively infiltrate any tissue, they might regulate preferential concentrations of active estrogens at certain critical sites, particularly at their sites of contact with specific target cells.

The rate of estrone to 17β-estradiol conversion by the blood mononuclear cells was not significantly different in men, in premenopausal women, and in postmenopausal women; similarly, it was not influenced by the concentrations of steroid sex hormones or by precursors in the medium; it was thus to a large extent independent of the endocrine environment. The rate of estrone to 17β-estradiol conversion did not rise with the concentrations of glucose and insulin in the medium; the generation of NADPH by the hexose monophosphate shunt was thus not a rate-limiting step at normal glucose concentrations (19). Consequently, the rate of estrone to 17β-estradiol conversion appears to be a stable and characteristic parameter of blood mononuclear cell function.

In view of this stability, it is remarkable that the conversion of estrone to 17β-estradiol by the blood mononuclear cells was definitely lower in the patients with localized carcinomas. The lowest rates were found with the most localized or smallest tumors. Although a decreased conversion rate appeared to be present at the very beginning of the disease, it is impossible to know if it was primary or secondary. If primary this could indicate that certain blood mononuclear cells exert their cytotoxicity, especially against hormone-dependent tumors, through endocrine mechanisms, and it might be relevant that the conversion of estrone to 17β-estradiol in the patients with breast carcinomas was significantly lower than in the patients with other tumors. On the other hand, if secondary, the low estrone to 17β-estradiol conversion rate in patients with localized carcinomas might be a nonspecific consequence of the decrease in the percentage of active T-lymphocytes in patients with cancer (23); however, this would not explain the recovery of normal or even high conversion rates with the generalization of the tumor unless a distinct subpopulation of cytotoxic blood mononuclear cells was progressively activated in response to the spread of the disease. This distinct subpopulation might be the monocytes. Indeed, when human blood mononuclear cells are fractionated according to their ability to phagocyte iron and to form rosettes with sheep RBC, we find that the estrone to 17β-estradiol conversion by the monocytes is moderately to considerably higher than that produced by a corresponding number of T-lymphocytes (A. Borkowski and J. Wybran, work in progress). Our present finding that a significant correlation between the estrone to 17β-estradiol conversion rate and the number of monocytes became detectable precisely in the patients with generalized nonmammary carcinomas, i.e., the subjects with the highest conversion rate and the highest number of monocytes, would be consistent with this view. Consequently, the differences of conversion between the normal subjects and the various patients were probably attenuated by the heterogeneity of our blood mononuclear cells and could be amplified if subpopulations of the mononuclear cells were investigated separately.

Our studies might have 2 practical applications. First, from a prognostic point of view, in a patient with a presumed localized carcinoma, a high estrone to 17β-estradiol conversion rate should induce suspicion of generalization; on the other hand, a presumably normal subject with a high conversion rate appears less likely to have a small concealed malignant tumor. Secondly, from a therapeutic point of view, if a low conversion rate betrays a deficient immune response against the tumor the in vitro ability of certain drugs to increase the estrone to 17β-estradiol conversion by the patient’s mononuclear cells could be an index of their antitumoral efficiency.

REFERENCES


Estrone to Estradiol Conversion by Blood Mononuclear Cells in Normal Subjects and in Patients with Mammary and Nonmammary Carcinomas

A. Borkowski, M. Dosogne, P. Declercq, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/7/2174

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.