Serum Hormones and Lipoproteins in Benign Breast Disease

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Abstract

Seventeen young women with clinically confirmed mammary dysplasia and six age-matched controls were treated with α-tocopherol. Serum samples collected during the luteal phase of each woman at monthly intervals for the 4-month duration of the study were analyzed for serum luteinizing hormone, follicle-stimulating hormone, and prolactin concentrations by radioimmunoassay and for lipoprotein levels by a combination of precipitation, ultracentrifugation, and enzymatic techniques. Fifteen patients showed objective and subjective remission from disease. While prolactin levels did not change significantly, elevated levels of luteinizing and follicle-stimulating hormones were decreased to normal levels. Ratios of serum cholesterol to high-density lipoprotein cholesterol decreased; high-density lipoprotein and free cholesterol associated with low-density lipoproteins increased as a result of therapy. The results suggest that α-tocopherol may serve as an effective agent not only to treat patients with benign breast disease but also to normalize abnormal hormone and lipid levels in subjects at high risk for breast cancer.

Introduction

Current investigations indicate that patients with MD have an abnormal steroid milieu. Earlier work (17) suggested that women with MD have inadequate corpus luteal function, which may be secondary to an ovulatory disorder. Since LH is intimately related to the production and adequacy of the ovarian corpus luteum and since prolactin affects the breast tissue, information about these hormones might shed light on the abnormal ovarian steroid milieu observed (16) in these MD patients. Earlier studies concerning LH, FSH, and prolactin levels in urine or serum of MD patients show contradictions (7, 11, 12, 15). As early as 1955, Barclay et al. (2) found that LDL was elevated and HDL was depressed in women with breast dysplasia and six age-matched controls were treated with α-tocopherol. Serum samples collected during the luteal phase of each woman at monthly intervals for the 4-month duration of the study were analyzed for serum luteinizing hormone, follicle-stimulating hormone, and prolactin concentrations by radioimmunoassay and for lipoprotein levels by a combination of precipitation, ultracentrifugation, and enzymatic techniques. Fifteen patients showed objective and subjective remission from disease. While prolactin levels did not change significantly, elevated levels of luteinizing and follicle-stimulating hormones were decreased to normal levels. Ratios of serum cholesterol to high-density lipoprotein cholesterol decreased; high-density lipoprotein and free cholesterol associated with low-density lipoproteins increased as a result of therapy. The results suggest that α-tocopherol may serve as an effective agent not only to treat patients with benign breast disease but also to normalize abnormal hormone and lipid levels in subjects at high risk for breast cancer.

Since there is a 2- to 8-fold increase in breast carcinoma incidence in patients with MD (6), investigation into the etiology and therapy of this common disorder may provide an insight into etiology of breast carcinogenesis and could suggest potential protective measures. In this paper, we report the serum levels of LH, FSH, prolactin, and lipoproteins in MD patients before and after α-tocopherol (vitamin E) therapy.

Materials and Methods

Six control patients without underlying breast or other endocrine pathology and 17 patients with documented mammary dysplasia, on the basis of either mammography or previous biopsy, were enrolled in the study after giving informed consent. All subjects had regular menstrual cycles. During the first month, the patients received placebo tablets; during the second and third full months, the patients received DL-α-tocopherol acetate, 300 IU, 2 tablets/day. Serum samples were collected on the 21st day of the cycle when breast examinations were performed between 8:30 and 9:30 a.m.

Serum LH, FSH, and prolactin were determined by radioimmunoassay techniques. Commercial kits were obtained from Seralo Laboratories, Inc. (Braintree, Mass.), and Abbott Laboratories (North Chicago, Ill.) for the determination of LH, FSH, and prolactin, respectively, and their protocols were followed for performance of assays.

VLDL and LDL were isolated together by precipitation with heparin and MnCl₂ by established methods used in lipid research clinics. The HDL₂ and HDL₃ fractions were isolated by sequential preparative ultracentrifugation of the supernatant at 1.125- and 1.21-g/ml density in a Beckman Model L2-50 ultracentrifuge (8). The total and free cholesterol in the lipoprotein fractions were measured by the enzymatic method of Allain et al. (1) using a reagent kit from Biodynamics Division of Boehringer-Mannheim Company. Serum vitamin E concentrations were determined by a well-established procedure (5).

Statistical analysis was performed using Student's t test. The study was carried out in a double blind fashion. Clinical response to therapy was graded by both subjective (patient) and objective (physician) analysis. Subjective improvement at each examination was noted utilizing our previous criteria (10). Objective changes were recorded by a single examining physician. Criteria included overall tenderness, size, and location of the lesions and measurement of discrete lesions when detected.

Results

The pretreatment mean serum vitamin E level in the controls was 1.48 ± 0.31 (S. E.) mg/dl which significantly increased to 2.59 ± 0.48 mg/dl after treatment (p < 0.05). The pretreatment mean vitamin E level in the patients was 1.14 ± 0.04 which significantly increased to 2.33 ± 0.17 mg/dl (p < 0.001). Of the 17 patients, 9 showed very good response, 6...
showed fair response, and 2 showed no response to therapy. Serum chemistry profiles of both controls and patients did not change during the therapy. Placebo did not significantly alter the pretreatment hormone or lipoprotein levels.

Two controls (Subjects 3 S.C. and 19 M.S.) and 2 patients (Patients 7 A.S. and 1 M.G.) had abnormally elevated LH values which were decreased by treatment to almost normal levels (Table 1). If these abnormals are excluded from the study population, then there is no significant difference in the LH concentrations of controls and patients before or after therapy. If included, vitamin E appears to decrease the LH values in both controls and patients, but not with statistical significance (at p < 0.05). However, vitamin E decreases abnormally elevated LH levels in controls and patients, whether the patients are clinically good or nonresponders to therapy.

In the patients, the serum FSH concentrations (Table 1) changed from 4.5 ± 0.4 to 4.3 ± 0.4 mIU/ml after therapy. In controls, the levels changed from 7.7 ± 1.8 to 4.1 ± 1.3 mIU/ml. The pattern present with LH was seen in these measurements. Normal levels (2 to 8 mIU/ml) in luteal phase) present in all the patients were not affected, but elevated levels found in 2 controls (Subjects 20 V.A. and 28 S.W.) were decreased from 8.2 and 12 to 5 and 7 mIU/ml, respectively, as a result of therapy.

The prolactin levels (Table 1) of controls and patients were within normal range (0 to 25 ng/ml), and vitamin E did not significantly change the levels. One control (Subject 20 V.A.) had persistently abnormal levels (152, 174, 134, and 143 ng/ml during her 4 visits) of prolactin before and after therapy without the presence of detectable tumors by physical examination, neurological testing, visual field examination, and polytomographs of the sella turcica.

While the total serum cholesterol did not change in controls or patients, the ratio of total serum cholesterol to HDL cholesterol (Table 2) decreased from 5.3 to 4.3 (p < 0.05) in the patient group that had ratios higher than those of controls (3.9; p < 0.1) as a result of vitamin E therapy. HDL2 cholesterol levels (Table 2) did not change significantly, but HDL3 cholesterol concentrations increased from 21 ± 3 to 26 ± 1 mg/dl (p < 0.05) in the patients only. More dramatically, VLDL plus LDL free cholesterol increased significantly (p < 0.02) in both patients (38.3 ± 3.4 to 50.9 ± 7.3 mg/dl) and controls (35.5 ± 6.25 to 57.3 ± 7.2 mg/dl) (Table 2).

### Discussion

Sex hormones, iodine-containing agents, and diuretics have been widely used to treat mammary dysplasia, but they are not without side effects. Our study demonstrates that a nontoxic, noncaloric dietary factor, vitamin E, is capable of producing clinical improvement in 15 of 17 patients treated, with no demonstrable side effects.

Mirabile et al. (15) and Malarkey et al. (11) reported normal urinary and serum LH concentrations in subjects with benign and malignant breast disease. However, while Manuilova and Pschenichnikova (12) found low urinary LH secretion, Golinger et al. (7) found elevated serum LH levels in MD patients. Our results (Table 1) are similar to the findings of Golinger et al. (7). Elevated LH may have a role in the pathogenesis of mammary dysplasia. Our study shows that vitamin E is an

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Table 1

<table>
<thead>
<tr>
<th>Subjects</th>
<th>LH (mIU/ml) Before therapy</th>
<th>LH (mIU/ml) After therapy</th>
<th>FSH (mIU/ml) Before therapy</th>
<th>FSH (mIU/ml) After therapy</th>
<th>Prolactin (ng/ml) Before therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 6)</td>
<td>35 ± 11</td>
<td>19 ± 4</td>
<td>7.7 ± 1.8</td>
<td>4.1 ± 1.3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>3 S.C.</td>
<td>61</td>
<td>37</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>19 M.S.</td>
<td>77</td>
<td>7</td>
<td>9.2</td>
<td>5</td>
<td>152</td>
</tr>
<tr>
<td>20 V.A.</td>
<td>12.0</td>
<td>7.0</td>
<td>18 ± 2.5</td>
<td>18 ± 2.7</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>28 S.W.</td>
<td>38 ± 15</td>
<td>22 ± 3</td>
<td>4.5 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>Patients (n = 17)</td>
<td>82</td>
<td>35</td>
<td>252</td>
<td>26</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>7 A.S.</td>
<td>252</td>
<td>26</td>
<td>21 ± 4</td>
<td>21 ± 4</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>1 M.G.</td>
<td>20 ± 4</td>
<td>21 ± 4</td>
<td>21 ± 4</td>
<td>21 ± 4</td>
<td>21 ± 4</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* n = 5 since the abnormal values (152 and 143) of one control were omitted in calculating the mean.
* If the 2 abnormal controls, 3 S.C. and 19 M.S., are omitted from calculation.
* Nonresponder to therapy.
* Good responder to therapy.
* If the 2 abnormal patients, 7 A.S. and 1 M.G., are omitted from calculation.

Table 2

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Serum cholesterol:HDL cholesterol</th>
<th>HDL2 cholesterol (mg/dl of total cholesterol)</th>
<th>HDL3 cholesterol (mg/dl of free cholesterol)</th>
<th>VLDL + LDL free cholesterol (mg/dl of free cholesterol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 4)</td>
<td>3.9 ± 0.1</td>
<td>4.2 ± 0.7</td>
<td>23 ± 9</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>Patients (n = 11)</td>
<td>5.3 ± 0.5</td>
<td>4.3 ± 0.4</td>
<td>16 ± 4</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

* Mean ± S.D. The differences in the means (see text) are statistically significant; of the 11 patients analyzed, only one did not respond to therapy.
effective therapeutic agent in normalizing the elevated serum LH levels in controls or MD patients, although the mechanism by which such reduction takes place is unknown. A similar pattern is observed with FSH levels.

It has been suggested (13) that excessive prolactin levels may cause or aggravate mammary dysplasia. Our current study shows that serum prolactin concentrations of patients were similar to those of normal controls, although it appears that prolactin levels of patients are tending towards the lower limits of normal range. The relatively lower levels of prolactin in our patients suggest that prolactin insufficiency may be involved in the genesis of the disease.

The HDL may play important roles as carriers of growth inhibitors or as potentiaters of immunity. Since the lipoproteins transport not only lipids and proteins but also a multitude of vitally important cofactors, vitamins, hormones, and carbohydrates, alterations in any one or several of these, in relation to the whole complex, may be involved in the etiology of cancer. Our study shows that vitamin E therapy alters the serum cholesterol:HDL cholesterol ratios as well as HDL levels favorably in MD patients. The patients had ratios (5.3) higher (p<0.01) than did controls (3.9) before therapy; vitamin E normalized the ratios, suggesting a decrease in the risk for atherogenesis. Although the HDL cholesterol increased, the increase was more in the HDL3 fraction than in the HDL2 fraction. Increased HDL is known to afford protection against cardiovascular diseases. Low levels of HDL present in cancer patients and their relatives increase after surgical removal of tumor or treatment; whether an increase in HDL or HDL3 will provide protection against carcinogenesis in MD patients is unknown. At present, all the controls and subjects show an increase in the free cholesterol levels of VLDL plus LDL. Free cholesterol rather than esterified cholesterol enters and leaves cells freely and is easily converted into other steroid molecules. Increase in its levels due to vitamin E would suggest a beneficial effect.

In summary, our current investigation has confirmed our previous findings that vitamin E seems to be a safe and rational therapy for MD. In addition, this therapy seems to normalize serum abnormal LH or FSH concentrations without altering prolactin levels. The therapy also appears to alter the lipoprotein compositions favorably. The transformation of normal cells into neoplastic cells, their survival, and subsequent proliferation to form a tumor is a complex problem probably mediated by several factors. Vitamin E normalized hormonal and lipoprotein levels in MD patients and may have similar effects in other subjects at high risk for cancer.

Acknowledgments

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References

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