Cholesterol and Cholesterol Epoxides in Nipple Aspirates of Human Breast Fluid

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ABSTRACT

In nipple aspirates of breast fluid from nonpregnant healthy women, cholesterol and cholesterol epoxide levels were determined with gas-liquid chromatography and mass spectrometric techniques. Cholesterol levels were found to be elevated above plasma levels averaging 2200 ± 1995 (S.D.) mg/dl and showing progressive increases in mean breast fluid cholesterol levels with advancing age, averaging 187, 1957, and 3554 mg/dl in women of age groups 20 to 29, 30 to 39, and 40 to 49 years, respectively. Cholesterol epoxide was detected in a significant number of women who yielded high levels of breast fluid cholesterol. Cholesterol epoxide has been reported by other workers to have transforming activity for embryo hamster cells and to be carcinogenic in animals. The findings lend support to our hypothesis and observation that the human breast secretes mutagenic and cancer-promoting substances which may have relevance in studies of the etiology of benign breast disease and cancer.

INTRODUCTION

In previous studies, we demonstrated that chemical substances of exogenous and endogenous origin are secreted and concentrated by the nonlactating breast. We propose the hypothesis that certain chemical substances, secreted and accumulated by the breast, might initiate and promote actions in the breast epithelium that play an etiological role in the pathogenesis of benign and malignant breast disease (17-19).

Among the substances detected in nipple aspirates of breast fluid, cholesterol is a major component. We present data on cholesterol levels and on metabolically activated derivatives of cholesterol, cholesterol α- and β-epoxide. Cholesterol α-epoxide is a potent transforming agent known to damage DNA. Cholesterol epoxides have been reported by some investigators to be carcinogens (2, 10, 14, 16). Our findings indicate that high levels of cholesterol and significant levels of cholesterol α- and β-epoxide are present in the breast fluid of many women and suggest that they may be a significant factor in the pathogenesis of benign breast disease and cancer.

MATERIALS AND METHODS

Samples of breast fluid were obtained from 150 adult nonlactating, nonpregnant women by the nipple aspiration technique described previously (19). The women, 20 to 70 years old, attended the breast screening clinic at the University of California, San Francisco. Women who had had breast cancer were excluded from the study. Approximately 20 to 30 µl of breast fluid were obtained from a single aspiration in each subject.

Cholesterol levels in breast fluid of 150 women were determined by GLC using a Varian Model 3700 gas chromatograph and the method of Blomhoff (3). In 72 of the 150 women, plasma levels were also measured. In an additional separate group of 37 women, concentrations of total and free cholesterol and their α- and β-epoxide content were determined by the method of "inverse isotope dilution analysis" developed by Gruenke et al. (8, 9), with combined GLC-mass spectrometry using deuterium-labeled internal standards of cholesterol and cholesterol epoxides.

Instrumentation. The basic instrument consists of an Infotronics Model 2400 gas chromatography interfaced with an AEI MS-12 mass spectrometer via a molecular separator. The modification of the system for selected ion recording has been described previously (9). The gas chromatograph uses 2-m × 2-mm glass U-tube columns with 1% OV-1 on Chromosorb W AW DMCS (Supelco) at 240° isothermal and a helium flow rate of 25 ml/min. The retention time of the trimethylsilyl derivative of cholesterol was 3.0 min, and that of the epoxide derivative was 4.2 min.

Sample Preparation. To breast fluid samples (10 to 100 mg) were added 10.5 µg of cholesterol-2,2,4,4,6-d5 (7) and 1.68 µg of cholesterol-5α,6α-epoxide-2,2,4,4,6-d5 in 100 µl of heptane. The purity of these compounds was tested on thin-layer chromatography and by GLC in which single peaks were identical with those of unlabeled reference samples. Sterol esters were hydrolyzed by adding 2 ml of 95% ethanol containing 0.12 ml of 33% aqueous KOH and by heating at 55° for 15 min. Samples were worked up by adding 2 ml of water and 2 ml of benzene. The benzene layer was then removed, and the samples were evaporated to dryness. Trimethylsilyl ethers were prepared by adding 10 µl of pyridine and 50 µl of N,O-bis(trimethylsilyl)trifluoroacetamide and allowing the samples to stand at room temperature overnight.

Analytic Procedure. Since the electron impact mass spectra

1 Supported in part by USPHS Grant P01 CA 13556-09 from the National Cancer Institute, Bethesda, Md. Presented in part at the Fourth International Symposium on the Prevention and Detection of Cancer, London, July 26 to 31, 1980.

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Received September 12, 1980; accepted March 19, 1981.

3 The abbreviation used is: GLC, gas-liquid chromatography.

of the trimethylsilyl derivatives of cholesterol (M⁺, m/z 458 and m/z 463 for the d₅ compound) and cholesterol epoxide (M⁺, m/z 474 and m/z 479 for the d₆ compound) show prominent (10 to 30% of the base peak) molecular ions, these ions were chosen for monitoring. Standard curves were obtained by using solutions with known ratios (from 0 to 100 for cholesterol and from 0 to 5 for cholesterol epoxide) of compound to d₅ standard. The data were converted to mg/dl and μg/dl for cholesterol and cholesterol epoxide, respectively.

RESULTS

Cholesterol. Cholesterol levels in 150 individual breast fluid samples ranged from virtual absence to as high as 12,000 mg/dl; the mean was 2,200 ± 1,995 (S.D.) mg/dl (Chart 1). There was considerable variation between cholesterol levels in breast fluid and in plasma of the 72 individual women in whom both were measured. In many women, breast fluid levels were either similar or slightly lower than plasma levels; in others, cholesterol levels were markedly increased over plasma levels. When grouped according to age, there was a progressive increase in mean cholesterol levels, ranging from 187 ± 182 mg/dl in 20- to 29-year-old women to a high of 3554 ± 2073 mg/dl in 40- to 49-year-old women. In the over-50-year age group, the mean level was 2100 ± 1796 mg/dl. Corresponding mean plasma levels showed only slight (and expected) increase with advancing age (Table 1).

In 14 women, we determined the proportion of free and total breast fluid cholesterol; cholesterol esters averaged 32% and ranged from 0 to 78.6%.

Cholesterol α- and β-Epoxide. Cholesterol epoxide levels were determined in 37 breast fluid specimens, total cholesterol and epoxide in 20 specimens and free cholesterol and epoxide in 18 specimens. Seventeen of the 37 specimens showed measurable quantities of cholesterol epoxide. The individual values for cholesterol epoxide are shown in Table 2 according to varying range levels of free and total cholesterol in breast fluids. Concentrations of cholesterol epoxide in individual specimens ranged from 0 to 31,500 μg/dl. Fifty-five % of fluids in the 37 women studied contained no detectable cholesterol epoxides. There was a tendency for the greatest concentrations of epoxide to be found in those breast fluids that had high levels of either free or total cholesterol. Complete mass spectra of cholesterol epoxides from breast fluid, as the trimethylsilyl derivative, were shown to be identical with those of similarly derivatized authentic cholesterol α- and β-epoxides (Charts 2 and 3).

The 2 samples having the highest concentration of cholesterol epoxides and the next 3 highest samples combined were reanalyzed using a gas chromatography column which partially resolves the α and β isomers [1% SP 2250 (Supelco) column at 250°; retention time, 7.4 and 7.1 min, respectively]. In each case, both isomers were observed with the α isomer predominating.

We do not believe that the epoxide in breast fluid arises by the autooxidation of cholesterol during the analytical procedures, because concentration of epoxide did not increase in the samples tested at different times of storage at 5°, sometimes for as long as 7 to 10 days. Also, extracts of different breast fluid samples with differing concentrations of cholesterol processed in batches under identical conditions showed widely differing amounts of epoxide, and over one-half of the extracts contained none.

DISCUSSION

We have demonstrated here that cholesterol is a significant component of breast fluid which progressively increases in concentration from young adult life to middle age, although the concentrations vary widely among individuals. In contrast to our results, Wynder and Hill (24) did not find breast fluid cholesterol levels to be elevated above plasma levels in nipple aspirates from 8 premenopausal women; their samples of breast fluid may have been from younger women. Other studies (5, 13) have linked the source of cholesterol in fluid of the nonlactating breast ducts to transfer of cholesterol from plasma or its synthesis by the breast epithelium. It is probable that
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Cholesterol is also released through the breakdown of exfoliated epithelial and foam cells.

Our studies demonstrate the presence of cholesterol epoxide in 17 of 37 (45%) breast fluids examined for this substance from presumptively healthy women. There is evidence that cholesterol epoxide in breast fluid is most probably formed from cholesterol by enzymatic action (1, 4, 12, 22, 23). Recent studies indicate that many tissues contain cholesterol epoxides and hydrases (13). Gray et al. (6) found cholesterol epoxide in human serum; the highest levels were associated with elevated serum cholesterol levels. We found a similar association in breast fluid samples.

The possible carcinogenicity of cholesterol and its metabolites has been a topic of controversy for years (10). Earlier work showed that sarcomas and other tumors could be produced by injecting or feeding cholesterol and cholesterol epoxides (2). Black et al. (4, 15) found that cholesterol epoxide was formed in mouse and human skin by UV irradiation; they believe that carcinomas arising in mouse skin after such exposure are a result of cholesterol epoxide formation. More recently, Smith and Kulig (21) suggest that the previous uncertainty associated with the carcinogenicity of cholesterol and its derivatives may reflect the lack of information about some only recently discovered aspects of cholesterol metabolism. Recent reports indicate cholesterol epoxide as being nonmutagenic in the Ames test (13, 22). However, Kelsey and Pienta (14) found cholesterol epoxide to be as potent an agent in the embryo hamster transformation system as the known carcinogen 3-methylcholanthrene. Of additional significance is the report of Parson and Goss (16) that cholesterol epoxide induces chromosome damage and stimulates DNA repair synthesis in human fibroblast cultures. When viewed with earlier reports of its carcinogenicity, these findings indicate that cholesterol epoxide can damage DNA, and they offer important support for its potential carcinogenicity.

Our finding of cholesterol epoxide and high levels of cholesterol in the breast fluid of many women raises the possibility that these substances may play a role in the pathogenesis of benign breast diseases and breast cancer. We have noted that the nonlactating breast gland is unique among glandular organs in that it retains and concentrates its secretions resulting in the exposure of the epithelium to potentially harmful substances (18). Our findings suggest that the presence of high levels of cholesterol and cholesterol epoxides in breast secretions should be further investigated, especially in relation to the effects on breast epithelium and to the possible influence of nutritionally derived animal and plant sterols. It is of interest that Schaffner et al. (5) have found high concentrations of cholesterol epoxides in prostatic gland secretions for which they postulate a similar carcinogenic potential.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Trudy Beelen and Lynn Mason in the collection and analysis of breast fluid specimens.

REFERENCES


JUNE 1981 2565

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