Mutagenic Activity in Nipple Aspirates of Human Breast Fluid

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Abstract

The Ames Salmonella mutagenesis test was applied to nipple aspirates of breast fluid from 456 women attending breast-screening clinics. Positive tests for presumptive mutagenic substances were found in 6.7% of the women tested. These findings support our hypothesis that mutagenic substances reach the breast epithelia and may have etiologic implications in the pathogenesis of benign disease and breast cancer.

The nonlactating breast is unique among the secretory glands of the body in that its secretions are concentrated and metabolized within the gland (9—12). We have found many exogenous substances, including nicotine, fatty acids, technetium-99, and immunoglobulins, secreted and concentrated in breast fluid obtained by nipple aspirates from adult nonlactating women (9—13). We have proposed that this process provides a means whereby cancer-initiating and -promoting substances may reach the breast epithelium (7, 8). To test the hypothesis, we have used the Ames Salmonella mutagenesis test on nipple aspirates of breast fluid. Our preliminary results show that presumptive mutagenic substances are secreted into the breast fluid of some women.

We obtained 612 breast fluid samples by nipple aspiration described previously (12) from 456 women attending the breast clinic at the University of California (San Francisco) and the Breast Screening Center of Northern California and Merritt Hospital, Oakland, Calif. A clinical and epidemiological questionnaire was completed for each woman.

Breast fluid samples were stored in capillary tubes at —35° until the Ames Salmonella mutagenesis test (1) was conducted. The sample fluid was diluted into 100 μl of 50% dimethyl sulfoxide in distilled water for effective sterilization. The quantity of fluid available ranged from 5 to 50 μl. These small volumes permitted only one test per sample specimen. The entire sample was tested on a single plate after mixing with rat liver microsomal S-9 homogenate and one of the Ames histidine-dependent Salmonella tester strains (1). The samples were dependent. We used TA 1538 as the primary tester strain because of its broad spectrum of sensitivity to mutagens and its low rate of spontaneous revertant mutations.

Because histidine present in samples of body fluid can raise apparent spontaneous revertant mutation rates, we determined the free histidine level of breast fluid samples with an amino acid analyzer. The free histidine values ranged from 0.14 to 10.4 nmol/breast fluid sample with a mean of 2.04 ± 2.86 for 11 randomly selected specimens. As there are 100 nmol of histidine normally present in the top agar used in the Ames test, our values averaged only 2% and not more than 10% of increased histidine. We found that to increase the number of spontaneous revertant mutations above the average values normally found in control plates required at least 200 nmol of histidine in the top agar. The amount of histidine present in breast fluid, therefore, did not contribute significantly to the spontaneous revertant rate.

The spontaneous revertant control values were derived from 107 tests during the period of our study. A positive test for presumptive mutagens in breast fluid was set as being 2 S.D. above the mean of the spontaneous revertant control values. The mean and 2 S.D. of the controls were 26 ± 14 revertant colonies/plate. Most breast fluids gave revertant counts comparable to those of the controls, but 35 of 612 fluids (5.7%) were significantly elevated above this level, ranging from 41 to 376 revertant colonies/plate. About one-third of the 35 breast fluids were elevated 3 or more S.D. above the mean. There was no relationship between volume of breast fluid and positivity of the Ames test.

Breast fluid was positive in one or both breasts in 31 of 456 women (Table 1). In 152 women who yielded fluid from both breasts, 4 had positive Ames tests in both fluid samples. From 304 women, fluid had been obtained from either the right or the left breast. Eighteen of 147 (12.2%) fluids from right breasts and 9 of 157 (5.7%) fluids from left breasts were positive.

Attempts to correlate clinical and epidemiological characteristics of the women with positive mutagenesis tests were not informative. However, we can report several interesting findings. The highest proportion of positive Ames tests was found in breast fluid of women over 40 years, averaging 12.4% positive compared to 4.3% positive for women under 40 years (p < 0.05). Two of the positive tests occurred in breast fluid of women taking a chlorinated phenothiazine (Eskatal), an anorectic containing prochlorperazine and dextroamphetamine. The highest Ames test (376 colonies) was found in breast fluid from one of these women. Prochlorperazine has been reported recently to become mutagenic in the Ames test after brief exposure to UV radiation (5). We also found one positive test in 1 of 12 women who had had a mastectomy for breast cancer (4).

These preliminary results show that presumptive mutagenic substances are secreted into the breasts of some nonlactating women. We will continue to evaluate the significance of these findings through clinical follow-up of patients with positive and negative Ames tests, repeat assays of the chemical nature of

1 Supported in part by USPHS Grant CA 13556-08.
2 To whom requests for reprints should be addressed, at 1699 HSW, University of California, San Francisco, Calif. 94143.
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the mutagenic activity, and efforts to detect biological effects of mutagen-positive fluid on breast epithelial and other types of cells.

We surmise the substances responsible for many of the positive Ames tests to be of endogenous origin, such as steroid oxidation products or peroxidated lipids, or to be derived from exogenous sources, such as drugs, hair dyes, chemicals in food, smoking products, or occupational and other environmental exposures. Exogenously derived mutagens that have been demonstrated to gain access to the body through ingestion, inhalation, and the skin (2, 4, 5) are also likely to be secreted into the breast glands.

References


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