Reversal of Gonadotropin-releasing Hormone Agonist Induced Reductions in Mammographic Densities on Stopping Treatment[1]

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Abstract

Previously, we described the reduction in mammographic densities that occurred in premenopausal women after 12 months on a hormonal regimen designed to be chemopreventive for breast (and ovarian) cancer consisting of a gonadotropin-releasing hormone agonist (GnRHA) plus low-dose add-back estrogen-progestin. We sought to determine whether the density reduction persisted with continuation of the regimen for 24 months, and, if so, whether the densities would return to baseline after the regimen was discontinued. Twenty-one women, 27–40 years of age ,with a 5-fold greater than normal risk of breast cancer, were randomly assigned in a 2:1 ratio to the treatment group (14 women) and to a control group (7 women). The percentage of mammographic densities, calculated as the proportion of the breast area on the mammogram containing densities, were assessed blindly using a computer-based threshold method at baseline, after 12 and 24 months of treatment, and at between 6 and 12 months after treatment was stopped. The previously described percentage of mammographic density reductions of 9.7% ($P = 0.012$) after 12 months of treatment were increased slightly to 11.4% ($P = 0.010$) after 24 months of treatment, but the additional change was not statistically significant. Ten of 11 treated women assessed at 24 months had reduced percentages of mammographic densities compared with baseline. Six to 12 months after completion of treatment, the mean percentage of mammographic density in the treated group was no different from that at baseline (mean decline of 2.0%; $P = 0.73$). The women in the control group had no statistically significant changes in densities over the period of the study. Reductions in mammographic densities engendered by the GnRHA plus a low-dose add-back estrogen-progestin regimen persist as long as the women receive treatment. The densities return to baseline when the women resume normal menstrual cycles. These results confirm that mammographic densities are influenced by ovarian function. Improved efficacy of mammographic screening is to be expected as long as a woman continues on such a regimen. Whether such a regimen is chemopreventive for breast cancer remains to be established, but the recent report on a randomized trial of use of GnRHA alone in premenopausal breast cancer cases showing a marked reduction in incidence of contralateral disease provides strong support for the hypothesis.

Introduction

A hormonal contraceptive regimen based on a GnRHA,[3] to suppress ovarian function, and low-dose add-back estrogen and progestin has been described previously (1). The regimen was designed to minimize exposure of the breast epithelium to estrogen and progestin, while preserving the beneficial effects of estrogen on bone and other disease risks and still preventing endometrial hyperplasia. Spicer *et al.* (2) described the predicted reductions in breast and ovarian cancer risk with such a regimen; lifetime breast cancer risk was predicted to be reduced by one-third if the regimen was used for 5 years and by >50% if used for 10 years.

The effects of the hormonal regimen after 1 year of use have been reported previously (1, 3). The GnRHA-based regimen used in this study used a once-a-month depot formulation of a GnRHA with add-back estrogen and intermittent progestin given p.o. The regimen was quite acceptable to these high-risk subjects, and a symptom questionnaire used to assess tolerance of the regimen showed that subjects had fewer symptoms after initiation of the regimen (1). Symptoms associated with the luteal phase of the menstrual cycle, commonly referred to collectively as premenstrual syndrome, were eliminated. The women on the hormonal regimen had statistically significant reductions in mammographic densities from their baseline mammogram to the mammogram taken after 12 months of treatment (3). The women on the contraceptive regimen gained slightly more weight on average than the controls between baseline and 1 year (2.4 *versus* 1.2 kg; $P = 0.63$). Adjusting for this nonsignificant weight gain made almost no difference to

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[3] The abbreviations used are: GnRHA, gonadotropin-releasing hormone agonist; EPRT, estrogen-progestin replacement therapy; ERT, estrogen replacement therapy.

the results. The decrease in mammographic densities was detected when a simultaneous pair-wise evaluation method was used (comparing baseline and year-1 mammograms in random order from each woman as a pair in a blinded fashion) by two radiologists (3), or when mammograms were read in a blinded fashion using an expert outlining method developed by Wolfe *et al.* (4), or when a computer-based threshold method was used (5).

The purpose of the study reported here was to determine whether there were additional reductions in density after 24 months of treatment and whether the reduction in density would persist or the densities would return to baseline after the hormonal regimen was discontinued.

Materials and Methods

The method of subject selection has been described in detail previously (1). In brief: 21 women, 27–40 years of age (average, 33.5 years) with a 5-fold greater than normal risk of breast cancer, were randomly assigned in a 2:1 ratio to the GnRHA-based regimen group (14 women) or to a control group (7 women). The risk assessment was based on having a first-degree female relative (mother or sister) with bilateral breast cancer before age 50 or a prior personal diagnosis of lobular carcinoma *in situ*. The eligibility criteria were: (*a*) premenopausal at 25–40 years of age with a 5-fold greater than normal risk of breast cancer; (*b*) no prior malignancy (other than lobular carcinoma *in situ*); (*c*) bone mineral density not ≥2 SDs below normal for age; (*d*) normal cholesterol levels; and (*e*) a normal physical and pelvic examination. The women agreed to use a nonhormonal contraceptive method during the study period. The study was approved by the University of Southern California Medical School Institutional Review Board, Los Angeles, California, and written informed consent was obtained from all participants.

Women in the hormonal-regimen group received: (*a*) 7.5 mg of leuprolide acetate depot (Lupron Depot; TAP Pharmaceuticals, Chicago, IL) by i.m. injection every 28 days; (*b*) 0.625 mg of oral conjugated estrogen (Premarin; Wyeth-Ayerst, Philadelphia, PA) 6 of 7 days every week; and (*c*) 10 mg oral medroxyprogesterone acetate (Provera; Upjohn, Kalamazoo, MI) for the last 13 days of every fourth 28-day cycle. Eight women reported hot flushes or vaginal symptoms and had their dose of conjugated estrogen increased to 0.9 mg (1). During the course of the study, a small daily dose of oral androgen (1.25 or 2.5 mg of methyl-testosterone) was added to the regimen to replace the ovarian androgen production blocked by the action of the GnRHA. The women in the control group received no hormones during the study period.

One woman was removed from the hormonal-regimen group after two cycles of treatment because of poor compliance. A second woman in the hormonal-regimen group had breast implants, and her mammograms were found unsuitable for inclusion in this mammographic aspect of the study. The remaining 12 women completed 12 months of treatment, 11 of them completed 24 months on the hormonal regimen, and four completed 30 months of treatment. The latter four women and five additional women who were treated for 24 months were followed for 36 months (*i.e.,* for 6 or 12 months after cessation of treatment). The control women had a second and a third mammogram 12 and 24 months after baseline.

Digitized cranio-caudal mammograms were used to study mammographic changes. We used a validated computer-based threshold method developed by our colleagues to determine the percentage of mammographic densities, as previously reported (5). The percentage of mammographic densities (*i.e.,* area of densities as a percentage of the breast area) were estimated separately for the left and the right breast, and the average % density for the woman was then calculated and used in all analyses presented here. All readings of mammograms were performed by one of us (G. U.), masked as to baseline or follow-up, and as regarded being from treated or control women. All mammograms were reread for this report; consequently, the results for baseline and 12-month follow-up differ slightly from those reported previously (5).

Table 1 Change in percentage of mammographic densities between baseline and follow-up in women treated with a GnRHA-based regimen and in control women

Treatment group	Treatment mo 12—baseline	Treatment mo 24—baseline	Posttreatment mo 6–12—baseline
GnRHA group			
1	−2.2%	−1.3%	15.7%
2	−4.7%		
3	−19.9%	−28.3%	−25.8%
4	−8.9%	−3.9%	
5	−34.0%	−28.1%	8.4%
6	−1.9%	−6.0%	−0.2%
7	−22.2%	−17.9%	−15.5%
8	−8.7%	−15.2%	−0.5%
9	11.2%	8.5%	19.3%
10	−4.3%	−13.1%	−7.2%
11	−20.4%	−19.4%	−12.4%
12	−0.5%	−1.1%	
Mean (SE)	−9.7% (3.5%)	−11.4% (3.5%)	−2.0% (5.0%)
P	0.012	0.010	0.73
Control group			
13	2.7%	−10.9%	
14	−12.2%	−2.1%	
15	−4.1%	−4.0%	
16	10.4%	7.0%	
17	−1.5%	−9.5%	
18	−5.8%	5.0%	
19	−11.6%	−3.3%	
Mean (SE)	−3.2% (3.0%)	−2.5% (2.5%)	
P^a	0.30	0.47	

[a] *P*, Wilcoxon's signed rank test differences within the group.

The statistical significance levels within each group of the differences between the baseline mammograms and the mammograms for the following years were evaluated using Wilcoxon's signed rank test (6). All statistical significance levels (*P*s) quoted are two-sided.

Results

Table 1 and Fig. 1 show the changes in percentage of mammographic densities from baseline to 12 and 24 months in the treated group and the changes (from baseline) of the mammograms 6 or 12 months after treatment was discontinued in the treated group. Table 1 also shows the changes in the percentage of mammographic densities in the control group at 12 and 24 months.

Compared with baseline, there was a mean reduction (±SE) in the percentage of mammographic densities of 9.7% (±3.5%; $P = 0.012$) and 11.4% (±3.5%; $P = 0.010$) after 12 and 24 months in the GnRHA treatment group, respectively. After 24 months of treatment, 10 of 11 women for whom information was available had reduced densities. Six to 12 months after completion of treatment, the mean percentage of mammographic density was no different from that at baseline (mean change, −2.0% ± 5.0%).

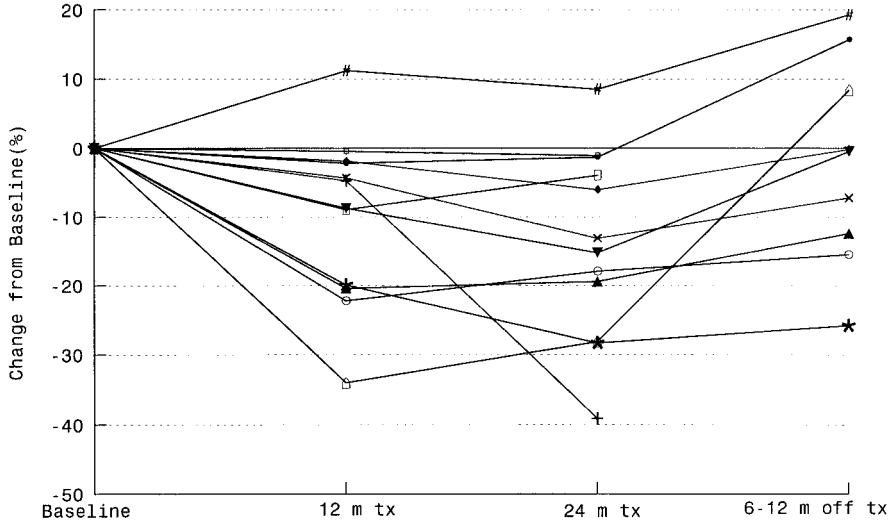

Fig. 1. Changes in the percentage of mammographic densities in women treated with a GnRHA-based regimen.

There was a mean change in the percentage of mammographic densities of −3.2% (±3.0%; $P = 0.30$) at 12 months and of −2.5% (±2.5%; $P = 0.47$) at 24 months in the control group. Neither of these changes was statistically significant.

Discussion

This study shows that most women on a GnRHA plus low-dose add-back hormonal regimen will show reductions in mammographic densities while they are on the regimen, and that these changes are reversed after cessation of the treatment.

Although we attempted to standardize the procedure for obtaining the mammograms, inspection of the projected area of the breast on different mammograms from individual women revealed that significant differences in the projection and/or compression of the breast had occurred. This is a common problem, not unique to this study. Absolute density measurements can be significantly affected by such artifacts. Expressing mammographic densities as a proportion of the breast area shown on the mammogram significantly reduces this problem, and is the measure of mammographic densities we have used in this report (percentage of mammographic density).

The percentage of mammographic densities are negatively associated with weight—for the same absolute density, heavier women will tend to have larger breasts so that the percentage of densities will be reduced. As is to be expected, there were some weight changes in both the intervention and control groups over the course of the study, but adjustment for such changes had little effect on the results shown in Table 1. We had no information on when in the menstrual cycle the baseline and off-study mammograms were obtained, but this is unlikely to be important (7).

The mean percentage of mammographic density 6–12 months after stopping use of the GnRHA-based regimen was no different from the mean percentage of mammographic density at baseline. However, the mean of close to no change conceals a wide range of apparent changes in individual women (see Table 1 and Fig. 1). We believe that this wide variability is an artifact caused by the lack of strict uniformity in the taking of mammograms (positioning and compression). In this regard, it is noteworthy that the two women (subjects 1 and 9) with the largest measured increases in percentage of density had almost no reduction (subject 1) or an actual increase (subject 9) in percentage of density while on GnRHA treatment; the most likely explanation of this is that the baseline mammograms were taken in a different manner, and inspection of the relevant mammograms is somewhat supportive of this interpretation, although it is not completely clear. Future studies will need to pay very close attention to obtaining standardized mammograms if reliable interpretation of individual changes are to be made.

It seems most likely that the described reductions in the percentage of mammographic densities are directly attributable to the reduced hormone exposure, and the return to baseline is directly attributable to the resumption of ovulation. However, although all women resumed regular menses during the follow-up, we do not know exactly when they started ovulating, and therefore cannot estimate directly how density changes were associated with the timing of resumption of ovulation.

Although there are no other randomized clinical trials to which we can directly compare these results, the results from this study are in accordance with other studies that examined the relationship between hormone levels and mammographic densities. First, mammographic densities decrease after menopause (8, 9). Second, there is compelling evidence from many observational studies that postmenopausal EPRT significantly increases mammographic density, and this has been confirmed in a placebo-controlled randomized trial (10). Third, the GnRHA-based regimen studied here mimics a bilateral oophorectomy with ERT with infrequent use of a progestin. Postmenopausal ERT does increase mammographic densities but only to a small extent, much less than does EPRT (10), so the reduction in densities seen in this study is as one would predict. If the progestin had been added every 28-day cycle or had been given continuously, one would have expected a much smaller reduction in densities or possibly no reduction at all.

The return to baseline densities after cessation of the regimen agrees with the opposite effect seen in a study of the effect of cessation of EPRT on mammographic density (11). In this latter study, 47 women who experienced a new or enlarging mass or a developing mammographic density while on HRT (the type was not specified; but, inasmuch as the study was done in 1995–1996, most were likely to have been on EPRT), 35 (75%) underwent a reduction in density or resolution of the new mass shortly after cessation.

The likely improvement in efficacy of screening mammography to be gained from the use of such a GnRHA-based regimen in premenopausal women has been emphasized by Feig (12), and this possibility is strongly supported by the studies of the effects of EPRT on mammographic screening in which EPRT use led to an increased incidence of interval cancers between screening mammograms (13, 14).

Mammographic densities have consistently been shown to be a strong breast cancer risk factor in epidemiological studies (9, 15–17). Factors that substantially reduce breast densities would, therefore, be expected to significantly reduce breast cancer risk. We have argued previously that, although the reductions in densities are reversed after cessation of the regimen, relatively short-term use of the regimen would still be expected to confer a significant lifelong reduction in breast cancer risk because the total amount of breast cell proliferation will be permanently reduced (2). The initial report from a randomized ZIPP trial of use of a GnRHA for treatment of premenopausal breast cancer showed a 40% reduction in contralateral breast cancer incidence in patients receiving the GnRHA (18). The addition, in the GnRHA regimen discussed here, of low-dose ERT with minimal progestin given only three or four times/year should only marginally affect this figure, because the breast cancer risk from ERT is low (19). Tamoxifen's breast cancer chemopreventive action in women without breast cancer was accurately predicted by its effect on preventing contralateral breast cancer in breast cancer patients. These results (18) are therefore most encouraging of the predicted breast cancer chemopreventive efficacy of the regimen discussed here.

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References

1. Spicer, D. V., Pike, M. C., Pike, A., Rude, R., Shoupe, D., and Richardson. J. Pilot trial of a gonadotropin hormone agonist with replacement hormones as a prototype contraceptive to prevent breast cancer. Contraception, *47:* 427–444, 1993.

2. Spicer, D. V., Shoupe, D., and Pike, M. C. GnRH agonists as contraceptive agents: predicted significantly reduced risk of breast cancer. Contraception, *44:* 289–310, 1991.

3. Spicer, D. V., Ursin, G., Parisky, Y. R., Pearce, J. G., Shoupe, D., Pike, A., and Pike, M. C. Changes in mammographic densities induced by a hormonal contraceptive designed to reduce breast cancer risk. J. Natl. Cancer Inst., *86:* 431–436, 1994.

4. Wolfe, J. N., Saftlas, A. F., and Salane, M. Mammographic parenchymal patterns and quantitative evaluation of mammographic densities: a case-control study. Am. J. Roentgenol., *148:* 1087–1092, 1987.

5. Ursin, G., Astrahan, M. A., Salane, M., Parisky, Y. R., Pearce, J. G., Daniels, J. R., Pike, M. C., and Spicer, D. V. The detection of changes in mammographic densities. Cancer Epidemiol. Biomark. Prev., *7:* 43–47, 1998.

6. Rosner, B. Fundamentals of Biostatistics, Ed. 5. Duxbury, Pacific Grove, California, 2000. pp. 338–343.

7. Ursin, G., Parisky, Y. R., Pike, M. C., and Spicer, D. V. Mammographic density changes during the menstrual cycle. Cancer Epidemiol. Biomark. Prev., *10:* 141–142, 2001.

8. Grove, J. S., Goodman, M. J., Gilbert, F., and Clyde, D. Factors associated with breast structure in breast cancer patients. Cancer (Phila.), *43:* 1895–1899, 1979.

9. Oza, A. M., and Boyd, N. F. Mammographic parenchymal patterns: a marker of breast cancer risk. Epidemiol. Rev., *15:* 196–208, 1993.

10. Greendale, G. A., Reboussin, B. A., Sie, A., Singh, H. R., Olson, L. K., Gatewood, O., Bassett, L. W., Wasilauskas, C., Bush, T., and Barrett-Connor, E. Effects of estrogen and estrogen-progestin on mammographic parenchymal density. Postmenopausal estrogen/progestin interventions (PEPI) investigators. Ann. Intern. Med., *30:* 262–269, 1999.

11. Harvey, J. A., Pinkerton, J. V., and Herman, C. R. Short-term cessation of hormone replacement therapy and improvement of mammographic specificity. J. Natl. Cancer Inst., *89:* 1623–1625, 1997.

12. Feig, S. A. Hormonal reduction of mammographic densities: potential effects on breast cancer risk and performance of diagnostic and screening mammography. J. Natl. Cancer Inst., *86:* 408–409, 1994.

13. Kavanagh, A. M., Mitchell, H., and Giles, G. G. Hormone replacement therapy and accuracy of mammography screening. Lancet, *355:* 270–274, 2000.

14. Mandelson, M. T., Oestreicher, N., Porter, P. L., White, D., Finder, C. A., Taplin, S. H., and White, E. Breast density as a predictor of mammographic detection: comparison of interval- and screen-detected cancers. J. Natl. Cancer Inst., *92:* 1081–1087, 2000.

15. Saftlas, A. F., and Szklo, M. Mammographic parenchymal patterns and breast cancer risk. Epidemiol. Rev., *9:* 146–174, 1987.

16. Boyd, N. F., Byng, J. W., Jong, R. A., Fishell, E. K., Little, L. E., Miller, A. B., Lockwood, G. A., Tritchler, D. L., and Yaffe, M. J. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J. Natl. Cancer Inst., *87:* 670–675, 1995.

17. Byrne, C., Schairer, C., Wolfe, J., Parekh, N., Salane, M., Brinton, L. A., Hoover, R., and Haile, R. Mammographic features and breast cancer risk: effects with time, age, and menopause status. J. Natl. Cancer Inst., *87:* 1622–1629, 1995.

18. Baum, M. Adjuvant treatment of premenopausal breast cancer with zoladex and tamoxifen: results from the ZIPP trial organised by the Cancer Research Campaign (CRC) Breast Cancer Trials Group. Breast Cancer Res. Treat., *57:* 30, 1999.

19. Ross, R. K., Paganini-Hill, A., Wan, P. C., and Pike, M. C. Effects of hormone replacement therapy on breast cancer risk: estrogen *versus* estrogen plus progestin. J. Natl. Cancer Inst., *92:* 328–332, 2000.

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