

Serum homocysteine levels in postmenopausal breast cancer patients treated with tamoxifen

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Abstract

Adjuvant treatment of breast cancer with tamoxifen may be associated with reduced risk of cardiovascular disease. Serum homocysteine level has been suggested to be a risk factor for cardiovascular disease influenced by estrogenic hormones. We evaluated a subset of postmenopausal women who had participated in a longitudinal, double-blind, randomized, placebo-controlled toxicity study of tamoxifen 10 mg orally, twice daily. Twenty-seven treated subjects and 37 placebo subjects had measurements of serum homocysteine levels made on previously frozen samples obtained at baseline and after 12 months. After treatment with tamoxifen, we found lower levels of serum homocysteine of borderline statistical significance. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Adjuvant therapy with tamoxifen is a major component of the treatment of operable breast cancer in postmenopausal women. While optimal duration of therapy has not been defined, low-risk (i.e. axillary node-negative) as well as high-risk (i.e. node-positive) patients appear to enjoy increased disease-free and overall survival with this treatment [1]. With earlier detection of lower stage breast cancer, a majority of postmenopausal women treated with tamoxifen

may be expected to survive for greater than 5 and 10 years.

As women age, cardiovascular diseases develop as the primary causes of morbidity and mortality [2], and thus any impact of adjuvant tamoxifen treatment on these processes is of significant concern. Recent data demonstrate apparent reductions in cardiovascular mortality [1], myocardial infarction [3] and hospitalization for heart disease [4] with tamoxifen treatment. Reduction in levels of several biochemical risk factors for cardiovascular diseases have been found with tamoxifen treatment: total cholesterol [5], LDL cholesterol [5], lipoprotein(a) cholesterol [6], fibrinogen [7] and platelet numbers [7].

Increased serum homocysteine levels are a risk factor for cardiovascular disease [8,9]. Genetically

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determined conditions of elevated levels [10] as well as acquired increases are associated with increased risk for premature cardiovascular disease. Differences in serum homocysteine levels in different estrogenic states in postmenopausal women [11] led to an evaluation of this factor with tamoxifen therapy [12]. The current study was conducted to investigate further the suggested reduction of serum homocysteine with tamoxifen therapy [12,13].

2. Patients and methods

2.1. Patients

We evaluated women who had participated in the Wisconsin tamoxifen study, a double-blind randomized, placebo-controlled, 2-year toxicity study of tamoxifen 10 mg orally, twice daily [14]. Patients in this study were 140 postmenopausal women, less than 65 years of age (range 46–64), who had a diagnosis of axillary node-negative breast cancer and who were disease-free. Details of requirements for eligibility have been reported previously [15]. After entry onto the study, which entered patients beginning in late 1986 until 1988, blood samples were obtained at baseline before tamoxifen treatment, and then at 3, 6, 12, 18 and 24 months. Only samples obtained at baseline and 12 months were evaluated in the current study due to availability of serum. All study subjects randomized to tamoxifen were subsequently found to have serological evidence of this drug and all subjects randomized to placebo were not found to have surreptitiously taken the drug.

2.2. Study sample population selection

The current study was designed and performed as a secondary investigation after investigations of other cardiovascular and osseous disease risk factors had been conducted using the obtained study blood samples. We evaluated samples of all subjects with available baseline and 12-month serum samples. There were 27 treated subjects of the originally randomized 70, and 37 of the randomized 70 placebo subjects with available samples.

None of these 64 subjects was taking corticosteroids, estrogens or lipid-lowering agents, and all had normal renal function at baseline. All fasting blood

samples were promptly centrifuged and then the serum was stored at -70°C . for periods of up to 7 years. The impact, if any, on homocysteine levels of such long-term storage of frozen specimens, is unknown, but we believe it's minimal (unreported data PEL). Specimens were mixed after thawing. Laboratory measurements were made on coded specimens, with the technicians blinded to the treatment condition.

3. Laboratory methods

Total serum homocysteine levels were determined by a modification of an automated procedure developed for the determination of total homocysteine in plasma [16].

The within assay coefficient of variation of this method is approximately 2% while the between assay coefficient of variation is $< 5\%$.

4. Statistical methods

While baseline and 12-month samples were determined to be available for 27 treated subjects and 37 placebo subjects, at some time in the study process the baseline sample or value for one treated subject was lost. The result for the 12-month evaluation for this subject was included in all analyses.

A single subject of the 64, a tamoxifen-treated subject, had baseline and 12-month homocysteine levels which were significantly above the upper limit of normal values of $15\ \mu\text{mol/l}$ (21.4 and $20.3\ \mu\text{mol/l}$, respectively). If this subject is included, the data are abnormally distributed using the Shapiro and Wilk statistic [17]; if this subject is not included, the data are normally distributed. Mean baseline and 12-month homocysteine levels were compared within each of the tamoxifen-treated and placebo subjects using a paired *t*-test. Mean baseline, 12-month, and the differences between these values were compared between the tamoxifen and placebo-treated groups using two-sample two-sided *t*-tests and Wilcoxon two-sample tests [17]. The relationship of the change in homocysteine level to the baseline value was assessed using a Spearman Rank correlation [17]. Data were analyzed using the SAS System [18].

Table 1

Comparisons of mean baseline and 12-month serum homocysteine values in tamoxifen and placebo groups^a

	Tamoxifen-treated mean (\pm SE)	Placebo mean (\pm SE)	<i>P</i> **	<i>P</i> ***
Baseline (μ mol/l)	9.42 \pm 3.1 (<i>n</i> = 26)	9.76 \pm 2.3 (<i>n</i> = 37)	0.61	0.32
12-month (μ mol/l)	8.93 \pm 2.9 (<i>n</i> = 27)	9.57 \pm 2.0 (<i>n</i> = 37)	0.30	0.06
Difference	– 0.43 \pm 0.26	– 0.19 \pm 0.29	0.65	0.41
<i>P</i> *	0.12	0.51		

^a **P* value from paired *t*-test for differences of baseline and 12-month values; ***P* value from two-sample *t*-test for differences between groups; ****P* value from Wilcoxon two-sample test for differences between groups.

5. Results

The study samples, tamoxifen (*n* = 27) and placebo (*n* = 37), did not differ in mean age, weight, body mass, exercise hours per week or hematocrit. In addition, these samples did not differ on any of these characteristics from the originally randomized groups of 70 each, of which they are subsets, nor from the sample of non-studied tamoxifen-treated subjects (*n* = 43) nor the sample of non-studied placebo subjects (*n* = 33). Periods of sample storage and freezer sites of storage were similar for the two groups of subjects.

When data for all studied subjects are included (Table 1), there were non-significant reductions and no significant differences between baseline and 12-month serum homocysteine values for the tamoxifen-treated group (*P* = 0.12) or the placebo group (*P* = 0.51). The baseline mean values did not differ for these two groups (*P* = 0.61), and the 12-month values did not differ (*P* = 0.30) by

two-sample *t*-tests. At baseline the two groups did not differ (*P* = 0.32) but had differences of borderline statistical significance at 12 months (*P* = 0.06) by the Wilcoxon two-sample *t*-test. Comparison of patient difference or changes from baseline were not significant by a two-sample *t*-test (*P* = 0.65) and a Wilcoxon test (*P* = 0.41).

A more suggestive picture is seen if results are analyzed with exclusion of the single tamoxifen-treated subject with significantly elevated baseline and 12-month values (Table 2). In this analysis there is a suggestion of a significant difference between the two groups in mean values at 12 months (*P* = 0.03), although the mean changes from baseline to 12 months for the groups are not statistically different (*P* = 0.65, two-sample *t*-test) (*P* = 0.48, Wilcoxon test).

The mean decreases in the two groups without the single high value subject data appear identical (Fig. 1).

Table 2

Comparisons of mean baseline and 12-month serum homocysteine values in tamoxifen and placebo groups with removal of data from one subject with significantly high values^a

	Tamoxifen-treated mean (\pm SE)	Placebo mean (\pm SE)	<i>P</i> **	<i>P</i> ***
Baseline (μ mol/l)	8.94 \pm 1.8 (<i>n</i> = 25)	9.76 \pm 2.3 (<i>n</i> = 37)	0.14	0.2
12-month (μ mol/l)	8.49 \pm 1.9 (<i>n</i> = 26)	9.57 \pm 2.0 (<i>n</i> = 37)	0.03	0.03
Difference	– 0.40 \pm 0.27	– 0.19 \pm 0.29	0.65	0.48
<i>P</i> *	0.16	0.51		

^a **P* value from paired *t*-test for differences of baseline and 12-month values; ***P* value from two-sample *t*-test for differences between groups; ****P* value from Wilcoxon two-sample test for differences between groups.

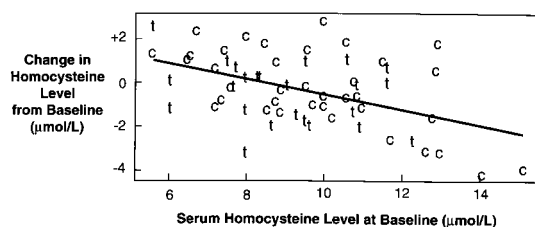


Fig. 1. The relationship of the mean changes in serum homocysteine levels from baseline to 12 months to the baseline levels in placebo (c) and tamoxifen-treated (t) subjects without inclusion of data from one subject with significantly high values. The regression lines for the relationship for both placebo (control) (c) and tamoxifen-treated (t) groups are superimposed (i.e. the same). The Spearman Rank correlation coefficient is 0.38 ($P = 0.003$). Thus a decrease in serum homocysteine levels of identical magnitude was seen in both placebo and tamoxifen treated groups. No explanations for this finding are obvious.

6. Discussion

While only selected subsamples of the entire population of patients in the Wisconsin tamoxifen study were investigated for the current report, there are no obvious differences in the studied tamoxifen and placebo groups that might bias or confound the measurements made or influence the interpretation of results.

The results of the current study do not show strong effects of tamoxifen treatment on serum homocysteine levels in breast cancer disease-free postmenopausal women. While some analyses suggest a difference in the change in homocysteine levels (Table 2), the differences are not significant. This analysis is limited by the study size and does not have high power to demonstrate that differences of the magnitude found were statistically significant (Table 1 and Fig. 1). The figure strongly emphasizes the uncertainty of the suggested decrease of homocysteine with tamoxifen.

The reasons the results here may differ from those of one earlier study [12] are multiple. That study strongly suggested that after 9–12 months of treatment serum homocysteine levels were significantly decreased by 20–30% with tamoxifen treatment [12]. The distribution of homocysteine levels in the population studied here is generally lower than in the previous study suggesting a demographically different population. Specifically, the absence of metastatic disease, the lower mean age in the group studied

here (58 versus 65) and dietary differences may be important. Homocysteine levels increase with age [19]. In the earlier study, the subjects with the highest levels of homocysteine at baseline experienced the greatest decreases with treatment. Some samples in the current study may not have been promptly centrifuged and frozen [which would be expected to increase homocysteine levels]. Another important difference in the current study is that the dose of tamoxifen was 10 mg twice daily; in contrast, in the earlier study, a 30 mg dose was used.

A more recent study [13] with a similar sized study sample, in which homocysteine measurements were done at baseline, 2 and 6 months, was able to demonstrate statistically significant decreases in homocysteine over time. The mean plasma levels of homocysteine, the standard deviation in values, and most importantly, the absolute level of decrease suggested with tamoxifen were all very similar to the values reported here. The study was statistically more powerful because of the three time point assessment design.

Because of the increasing data suggesting a relationship of homocysteine levels to heart disease [8] and because, in addition to breast cancer-afflicted women, healthy women are also being treated with tamoxifen in prevention studies, detailed understanding of the relationship of tamoxifen and homocysteine levels is important.

In summary, we have found no strong evidence that tamoxifen changes homocysteine levels in postmenopausal women. Because of possible beneficial effects on homocysteine levels, further research is warranted in controlled studies of larger populations.

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