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PLASMA LEVELS OF THE ATHEROGENIC AMINO ACID HOMOCYSTEINE IN POST-MENOPAUSAL WOMEN WITH BREAST CANCER TREATED WITH TAMOXIFEN

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Long-term treatment of breast-cancer patients with the anti-oestrogen tamoxifen has been found to be associated with reduced cardiovascular mortality. Plasma homocysteine is an independent risk factor for atherosclerotic disease, and its level is determined by folate and cobalamin status, and possibly also by oestrogen status. We measured the effect of tamoxifen on plasma homocysteine, serum cholesterol, serum cobalamin and serum and erythrocyte folate in 31 post-menopausal women with breast cancer. The plasma homocysteine level was decreased by a mean value of 29.8% after 9–12 months and by 24.5% after 13–18 months of treatment. Tamoxifen suppressed serum cholesterol by mean values varying between 7.2% and 17.6% after 3 to 19 months of treatment. There was no correlation between changes in plasma homocysteine and serum cholesterol. These findings suggest that the homocysteine-lowering effect of tamoxifen may contribute to the reduction of cardiovascular mortality observed in patients on adjuvant therapy with tamoxifen.

The synthetic anti-oestrogen tamoxifen was primarily used for treatment of advanced breast cancer, and is now widely used for adjuvant therapy (Early Breast Cancer Trialists' Collaborative Group, 1992). It is currently being investigated as a chemopreventive agent in women at high risk of developing breast cancer (Powles *et al.,* 1990).

In the Scottish trial on adjuvant tamoxifen, patients who received the drug for a 5-year period experienced a reduction of fatal myocardial infarctions of more than 50% compared to placebo-treated controls (McDonald and Stewart, 1991). In a recent report from Sweden, tamoxifen treatment was associated with a reduced incidence of hospital admissions for cardiac disease (Rutquist and Mattson, 1993).

Some biochemical effects of tamoxifen have recently been described, which may contribute to the cardioprotective effect. These include a moderate reduction in plasma cholesterol (Caleffi *et al.,* 1988; Love *et al.,* 1991; Dewar *et al.,* 1992) and lipoprotein(a) (Shewmon *et al.,* 1994), and changes in the oxidative defence system (Wiseman, 1994).

Elevated plasma levels of the sulphur amino-acid homocysteine (Hcy) represent a risk factor for cardiovascular disease (Ueland *et al.,* 1992). Various inborn errors of Hcy metabolism, termed homocystinuria, are associated with extremely elevated plasma Hcy, and these patients suffer from cardiovascular disease in early adolescence and even in childhood (Mudd *et al.,* 1989). Results from about 20 retrospective case-control studies (Kang *et al.,* 1992; Ueland *et al.,* 1992) and 2 recent prospective studies (Arnesen *et al.,* 1995; Stampfer *et al.,* 1992) suggest that also moderate hyperhomocysteinemia is associated with increased risk of premature cardiovascular disease.

The plasma Hcy level is partly a genetic trait (Reed *et al.,* 1991), but acquired states such as cobalamin and folate deficiencies and renal insufficiency may increase this level. There are indications that plasma Hcy is related to oestrogen status. Pre-menopausal women have lower plasma Hcy than men and post-menopausal women, (Boers *et al.,* 1983; Andersson *et al.,* 1992*a*), and plasma Hcy is decreased in pregnancy and in post-menopausal women receiving oestrogen substitution (Van der Mooren *et al.,* 1994; Andersson *et al.,* 1992*b*; Kang *et al.,* 1986).

Tamoxifen is considered to be an oestrogen antagonist, but acts as an oestrogen agonist on the levels of plasma proteins, like sex-hormone-binding globulin and thyroxin-hormone-binding globulin (Fex *et al.,* 1981; Sakai *et al.,* 1978), and on blood lipids (Love *et al.,* 1991; Dewar *et al.,* 1992).

The present study was undertaken to investigate whether tamoxifen acts on plasma Hcy level. In addition, we measured vitamins known to affect plasma Hcy, and serum cholesterol.

MATERIAL AND METHODS

Patients

Thirty-one post-menopausal breast-cancer patients who were scheduled to receive tamoxifen as adjuvant treatment (2 patients), for primary advanced disease (7 patients) or for relapsed disease (22 patients) were enrolled in the study. Their median age was 65 years (range 48–81 years). All patients received 30 mg tamoxifen daily except 2 who received 20 mg daily. One patient took 40 mg lovastatin o.d., and another patient received 8 g cholestyramine 3 times daily for hypercholesterolemia for 12 months before the start of tamoxifen treatment. Both continued with this treatment during the study.

Blood samples were taken from all patients before the start of tamoxifen therapy and at regular intervals during treatment. Fasting blood samples (10 ml in EDTA vacutainer tubes) were obtained by venipuncture and centrifuged within 30 min at 3,000 *g* for 5 min at 0–2°C. Plasma and serum fractions were stored at −20°C until analysis.

Biochemical methods

Total plasma Hcy and total cysteine levels were determined by a modification of an automated procedure developed for the determination of total Hcy in plasma (Fiskerstrand *et al.,* 1993). The precision (CV) of the method is about 2%.

Serum cobalamin was determined with a microparticle enzyme intrinsic factor assay run on an IMx system from Abbott (Abbott Park, IL). Serum and blood folate were assayed using the Quantaphase folate radioassay produced by BioRad (Hercules, CA). Cholesterol and creatinine were determined using the Chem 1 system (Technicon, Tarrytown, NY).

Statistical methods

Parameters were tested for normal distribution as raw data or after appropriate logarithmic transformation by use of Q-Q plot (Johnson and Wichern, 1982) and analysed using confidence intervals (Bulpit, 1987). Pre-treatment values of Hcy fitted a log normal distribution, while the changes in plasma

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Hcy and plasma Hcy/cysteine ratios during treatment (expressed as percentages of values before treatment) fitted a normal distribution well. Serum cholesterol, serum cobalamin, serum folate and erythrocyte folate expressed as percentages of values before treatment were best fitted to a log normal distribution. In addition to confidence intervals, plasma Hcy levels obtained before and during treatment were compared using non-parametric tests (Friedman non-parametric analysis of variance and the Wilcoxon matched pair sign rank test). Correlations were tested for using the Spearman rank correlation coefficient.

RESULTS

Pre-treatment values

The geometric mean value for total plasma Hcy before treatment was 12.4 μmol/l (range 5.13 to 32.9 μmol/l). Six patients had pre-treatment levels higher than 15 μmol/l which may be regarded as the upper normal limit (Ueland *et al.*, 1993). All patients had serum cobalamin within the normal range, and only one patient had serum folate (4.9 nmol/l) below normal; this patient also had the highest plasma Hcy (32.9 μmol/l). One patient had elevated serum creatinine, but her plasma Hcy level was within the normal range. Two patients had serum cholesterol levels above normal.

Previous investigations have shown that plasma Hcy is affected by folate and cobalamin status and by renal function (Ueland *et al.*, 1993). Before the start of tamoxifen treatment, total Hcy in plasma was negatively correlated with serum cobalamin ($r_s = -0.46$; $p < 0.01$) and erythrocyte folate ($r_s = -0.47$; $p < 0.02$), whereas the correlation to serum folate was not significant ($r_s = -0.14$; $p > 0.2$). There was no correlation between plasma Hcy and serum creatinine and between plasma Hcy and serum cholesterol.

Plasma Hcy during tamoxifen treatment

Mean plasma Hcy showed a small increase during the first 1–2 months after the start of tamoxifen treatment (Table I). Then total Hcy declined to about 70% of pre-treatment levels after 9–12 months of treatment. The Hcy/cysteine ratio showed a similar response. It is noteworthy that, at all time points from 5–8 months of therapy, 95% confidence intervals for mean relative values of plasma Hcy as well as the Hcy/cysteine ratio did not span the 100% value (Table I).

In all patients from whom we obtained plasma Hcy levels (n = 8) and plasma Hcy/cysteine ratios (n = 7) after 3–4, 5–8, 9–12 and 13–18 months of treatment, values were compared using the Friedman non-parametric analysis of variance. The test revealed a significant difference in total Hcy value ($p < 0.001$) and the Hcy/cysteine ratio ($p < 0.01$) between the different time points. When the changes in Hcy level and the Hcy/cysteine ratio were tested for each time point (Wilcoxon matched pair signed rank test), a significant decline was obtained at all time points except after the first 3–4 months (Table II). After 13–18 months of treatment, the plasma Hcy level had decreased in all patients.

The relative as well as the absolute reduction in total Hcy measured after 5–8 and 9–12 months of treatment was negatively correlated to the pre-treatment Hcy level (all r_s values between −0.93 and −0.68; $0.001 \leq p < 0.05$). Also, after 13–18 months of treatment, there was a trend—albeit not significant ($r_s = -0.29$)—towards a negative correlation between pre-treatment values and change of Hcy. One patient in this group was treated with the lipid-lowering drug cholestyramine that may increase plasma Hcy concentrations (Blankenhorn *et al.*, 1991). This patient had an atypical Hcy response to tamoxifen treatment. Her Hcy level was 27.6 μmol/l before the start of therapy and 26.5 after 13–18 months. When the data set from this patient was excluded (Fig. 1), the correlation approached statistical significance (relative values: $r_s = -0.48$; $p = 0.07$; absolute values: $r_s = -0.54$; $p = 0.04$).

Folate, cobalamin and cholesterol

Serum and erythrocyte folate concentration tended to increase during tamoxifen treatment (Table III), but the 95% confidence intervals of the mean spanned the 100% value. Serum cobalamin remained unchanged (Table III).

Serum cholesterol levels, given as percentages of pre-treatment values, are shown in Table IV. The mean suppression at the different time points varied between 7.2% and 17.6%, but the 95% confidence intervals spanned the control value of 100% in 2 of the 6 time intervals.

There was no correlation between individual suppression of serum cholesterol and suppression of plasma Hcy at any time interval.

DISCUSSION

The present study shows that treatment with tamoxifen for more than 3 months significantly reduced the plasma Hcy level. After 9–12 months, plasma Hcy as well as the Hcy/cysteine ratio were reduced by more than 25% (Tables I and II). The parallel decline in plasma Hcy and the Hcy/cysteine ratio implies that the cysteine level was not altered during tamoxifen treatment and demonstrates the selective suppression of the Hcy concentration.

The decrease in plasma Hcy induced by tamoxifen was correlated with the pre-treatment Hcy levels, so that the patients with the highest pre-treatment Hcy level showed the largest absolute decrease in plasma Hcy (Fig. 1). Thus, the patients who may be at high risk of developing coronary heart disease due to elevated plasma Hcy levels are those who may benefit most from tamoxifen treatment.

The average reduction in plasma Hcy induced by tamoxifen equals the difference in plasma Hcy in a population with vascular disease compared to healthy controls (Ueland *et al.*, 1992; Kang *et al.*, 1992). A recent prospective analysis including both men and women indicates that the relative risk for coronary heart disease may be increased by 40% for each 4 μmol/l increase in plasma Hcy, and may be somewhat higher for women than for men (Arnesen *et al.*, 1995). If this is the case, a reduction in plasma Hcy level, as observed during tamoxifen treatment in this study (Table I), may correspond to

TABLE I – PLASMA LEVELS OF HCY AND THE HCY/CYSTEINE RATIO DURING TAMOXIFEN TREATMENT EXPRESSED AS PERCENTAGE OF PRE-TREATMENT VALUES

Months of treatment	Hcy		Hcy/cysteine ratio	
	n	Mean (95% CI)	n	Mean (95% CI)
1–2	27	105.8 (95.6–115.9)	26	101.4 (93.7–109.0)
3–4	22	88.7 (77.7–99.6)	21	96.5 (88.5–104.5)
5–8	24	85.5 (75.8–95.1)	23	90.8 (88.2–99.3)
9–12	10	70.2 (54.5–85.9)	9	74.2 (60.9–87.5)
13–18	16	75.5 (65.9–85.5)	15	72.7 (58.8–86.7)
19+	9	78.6 (64.0–93.3)	8	73.2 (61.7–84.6)

TABLE II – *p* VALUES FOR THE COMPARISON OF PLASMA HCY AND THE PLASMA HCY/CYSTEINE RATIO BEFORE AND DURING TREATMENT[1]

Months of treatment	Number of patients	Hcy (*p*-value)	Number of patients	Hcy/cysteine ratio (*p*-value)
3–4	22	<0.05	21	$0.05 < p < 0.10$
5–8	24	<0.01	23	<0.025
9–12	10	<0.01	9	<0.01
13–18	16	<0.0001	15	<0.001
19+	9	<0.005	8	<0.01

[1]Wilcoxon matched pair sign rank test.

TABLE III – CONCENTRATIONS OF FOLATE AND COBALAMIN DURING TAMOXIFEN TREATMENT EXPRESSED AS PERCENTAGE OF PRE-TREATMENT VALUES

Months of treatment	Serum folate		Erythrocyte folate		Serum cobalamin	
	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)
1–2	27	87.7 (98.8–111.2)	20	100.1 (92.9–108.0)	26	98.5 (85.1–113.0)
3–4	21	115.0 (100.5–131.6)	19	102.0 (87.9–118.3)	21	96.5 (79.4–117.2)
5–8	23	107.1 (88.5–129.5)	18	118.0 (98.2–141.8)	23	93.0 (79.6–108.6)
9–12	10	134.3 (99.6–181.2)	6	143.1 (95.6–214.2)	9	90.0 (76.9–105.4)
13–18	16	114.9 (89.4–147.7)	13	116.6 (94.1–144.1)	16	85.9 (73.0–101.1)
19+	7	121.7 (77.2–191.9)	5	111.5 (78.2–158.8)	7	96.8 (78.0–120.1)

TABLE IV – CONCENTRATIONS OF CHOLESTEROL DURING TREATMENT EXPRESSED AS PERCENTAGE OF PRE-TREATMENT VALUES

Months of treatment	Number of patients	Cholesterol mean (95% CI)
1–2	25	91.9 (85.8–98.3)
3–4	22	90.1 (81.8–99.0)
5–8	24	85.6 (79.4–92.4)
9–12	9	90.9 (76.2–108.5)
13–18	16	92.8 (81.2–106.0)
19+	9	82.4 (74.0–91.9)

a reduction in cardiovascular disease of about 20–30%. Decreased plasma Hcy may therefore contribute to the reduced incidence of heart disease observed in patients receiving tamoxifen (McDonald and Stewart, 1991; Rutquist and Mattson, 1993).

In this and previous studies (Caleffi *et al.*, 1988; Love *et al.*, 1991; Dewar *et al.*, 1992), tamoxifen suppresses serum cholesterol by a mean value of 10–15%. In a recent analysis of 19 randomized trials, Holme showed that a 1% reduction in plasma cholesterol was associated with a 2.5% reduction in cardiovascular mortality rate (Holme, 1990). Accordingly, a 10–15% decrease in plasma cholesterol may account for a reduction in cardiovascular mortality of about 25–35%. If tamoxifen reduces the risk of fatal myocardial infarctions by more than 50%, as shown in the Scottish study (McDonald and Stewart, 1991), this suggests that additional factors may contribute to the effect. Suppression of plasma Hcy is a likely candidate.

The mean plasma Hcy in the breast-cancer patients was slightly higher than the level found in normal women of the same age (Andersson *et al.*, 1992*a*), and 6 out of 31 patients had a plasma Hcy value higher than 15 μmol/l, which may be regarded as the upper normal limit (Ueland *et al.*, 1993). Children suffering from acute lymphatic leukaemia have an elevated level of plasma Hcy compared with controls, but after chemotherapy plasma Hcy becomes normal (Kredich *et al.*, 1981; Refsum *et al.*, 1991). The possibility that the increase in plasma Hcy could reflect tumour burden should be considered. However, most of the patients enrolled in this study had a limited tumour burden, and there was no correlation between plasma Hcy and the tumour burden prior to tamoxifen treatment. We did not find any difference in Hcy response to tamoxifen treatment between patients with and without macroscopic disease. Therefore, tumour regression is not a likely mechanism behind the decrease in plasma Hcy during treatment in these patients.

At the present state of knowledge, at least 3 mechanisms for the Hcy-lowering effect of tamoxifen should be considered.

(1) Tamoxifen acts as an oestrogen agonist or as an oestrogen antagonist, depending on the species and target organ studied and the end point measured (Jordan, 1984). Reduction in plasma Hcy may be related to alteration in oestrogen status.

(2) Plasma Hcy is an indicator of intracellular folate function, and is negatively correlated to serum or erythrocyte folate

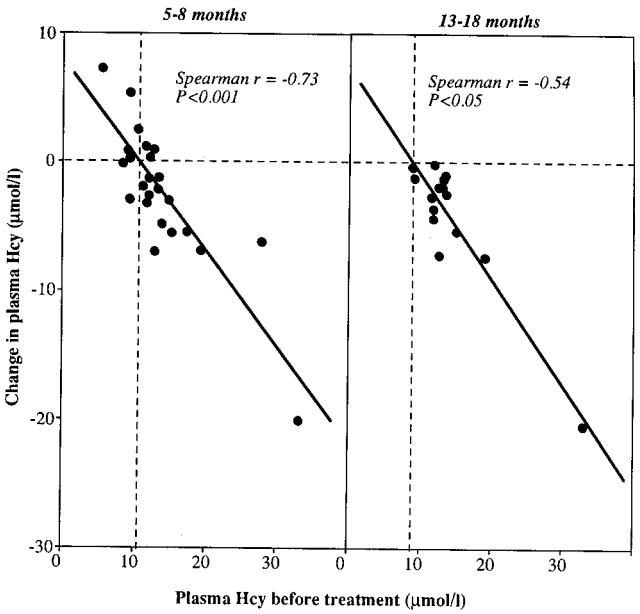

FIGURE 1 – Relation between plasma Hcy before start of tamoxifen treatment and change in plasma Hcy after 5 to 8 months of treatment (left panel) and 13 to 18 months of treatment (right panel).

(Ueland *et al.*, 1993). This relation existed prior to tamoxifen treatment for erythrocyte folate in the breast-cancer patients studied here. We also found a (non-significant) trend towards elevation of plasma as well as erythrocyte folate concentrations during treatment (Table III), suggesting that tamoxifen may exert its influence on Hcy level, at least partly, by influencing the folate homeostasis.

(3) Tamoxifen has antioxidant properties and suppresses hydrogen peroxide formation (Wiseman, 1994). We have recently shown that plasma thiols, including homocysteine interact via redox and disulphide exchange reactions (Mansoor *et al.*, 1992, 1993*a,b*, 1994). Conceivably, changes in the redox status brought about by tamoxifen may influence plasma Hcy levels.

In summary, tamoxifen treatment reduced the concentration of plasma Hcy by 25% in post-menopausal women with breast cancer. Patients with the highest pre-treatment homocysteine level showed the strongest Hcy response. The decrease in plasma Hcy may contribute to the reduced cardiovascular mortality in patients receiving tamoxifen.

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