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Original Paper

Effect of Tamoxifen on Lipoprotein(a) and Insulin-like Growth Factor-I (IGF-I) in Healthy Women

A. Decensi,[1] C. Robertson,[2] B. Ballardini,[1] D. Paggi,[3] A. Guerrieri-Gonzaga,[1] B. Bonanni,[1] L. Manetti,[1] H. Johansson,[4] A. Barreca,[4] D. Bettega[5] and A. Costa[1]

[1]FIRC Chemoprevention Unit; [2]Division of Epidemiology and Biostatistics, European Institute of Oncology, Via Ripamonti 435, 20141 Milan; [3]Laboratory of Clinical Chemistry, University of Milan; [4]Department of Endocrinology, University of Genoa; and [5]''Angelo Bianchi Bonomi'' Haemophilia and Thrombosis Centre, Milan, Italy

Studies in breast cancer patients have shown that tamoxifen decreases circulating levels of lipoprotein(a) (Lp(a)), an independent risk factor for premature coronary heart disease, and insulin-like growth factor-I (IGF-I), a promising surrogate biomarker for breast cancer. Since a common hormone regulatory pathway has been suggested for both biomarkers, we measured Lp(a) levels for 6 months in 68 healthy women participating in a chemoprevention trial of tamoxifen and correlated its changes with IGF-I. After 1 month, mean Lp(a) levels decreased by 23% with tamoxifen and increased by 6% with placebo ($P = 0.033$). No further change was observed after 2 and 6 months. Women with abnormal values at baseline (i.e. > 30 mg/dl) showed the highest reduction. The mean levels of IGF-I decreased by 23.5% with tamoxifen and remained stable with placebo, but the changes induced by tamoxifen in Lp(a) and IGF-I levels were uncorrelated. Our results support the observation that tamoxifen may be a suitable preventive option for women with multiple disease risk factors. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: tamoxifen, lipoprotein(a), chemoprevention, somatomedins, breast cancer
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INTRODUCTION

TAMOXIFEN, A selective oestrogen receptor modulator (SERM), represents the standard endocrine treatment for breast cancer [1] and can halve breast cancer incidence in at-risk women [2]. Although the striking results of the US prevention trial [2] were not confirmed in interim analyses of two smaller European trials [3, 4], tamoxifen seems to provide a suitable option for women who are at increased risk for breast cancer, particularly before the menopause.

Great interest has recently been focused on the role of lipoprotein(a) (Lp(a)) as a risk factor for coronary heart disease (CHD). Although similar to low-density lipoprotein (LDL) in core lipid composition and having B-100 as a surface apolipoprotein, Lp(a) is recognised as a distinct lipoprotein class that also contains the unique glycoprotein apo(a), which is disulphide-bound to apo B-100 [5].

Correspondence to A. Decensi, e-mail: adecensi@ieo.it,
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Because of the striking structural homology between apo(a) and human plasminogen, Lp(a) has been sought as a potential link between atherosclerosis and thrombosis [5]. In retrospective case–control studies using quantitative immunoassays, elevated Lp(a) levels, generally greater than 30 mg/dl, have been shown to confer an increased risk for CHD, in particular premature CHD (i.e. occurring in subjects by the age of 55–60 years) [5]. When appropriate storage conditions (− 70°C) were used or fresh specimens analysed, several prospective studies have confirmed that an elevated Lp(a) level is an independent predictor of CHD outcomes both in young men and women [6–9].

Tamoxifen has been reported to decrease Lp(a) levels in breast cancer patients [10] and in a small pilot study in healthy women [11]. Interestingly, a strong correlation between the changes in Lp(a) and in insulin-like growth factor-I (IGF-I), an important risk factor for premenopausal breast cancer [12], was observed in the latter study [11], suggesting a possible metabolic link between the two pathways. Given the

large interindividual variations in Lp(a) levels [5], we were interested in confirming these findings in a larger cohort. Moreover, we examined the time profile of Lp(a) modulation and whether the effect of tamoxifen is influenced by the initial Lp(a) level and could add important information in treatment decision-making for at-risk women, particularly with multiple disease risk factors.

PATIENTS AND METHODS

A consecutive cohort of 68 subjects were selected from an ongoing primary prevention trial of breast cancer for a 6 month safety study of the effects of 20 mg/day of tamoxifen on haemostasis and the lipid profile. Briefly, eligible women were aged between 35 and 70 years, had received a previous hysterectomy for non-malignant conditions and no contra-indication for tamoxifen use. When one or both ovaries had been preserved, postmenopausal status was defined as age > 50 years. The study received Institutional Review Board approval and all subjects provided written informed consent. A detailed description of the phase III trial has recently been published [3].

Although total cholesterol was the primary endpoint of the safety study, sample size was calculated assuming a 30% reduction in Lp(a) levels after 6 months of treatment from a mean ± standard deviation (S.D.) baseline level of 25 ± 12 mg/dl (power = 80%, two-tailed 5% significance level). Serum Lp(a) levels were measured at 0 (baseline), 1, 2, 4 and 6 months from randomisation. Unblinding was performed by the Data Safety and Monitoring Committee after analysis completion.

Fasting blood samples were taken between 0800 h and 1000 h. Serum aliquots were then centrifuged and stored at − 70°C until assayed in a single session. Serum Lp(a) levels were measured in duplicate by an immunoturbidimetric method [13], using a commercial kit purchased from Incstar Corp. (Stillwater, Minnesota, U.S.A.). The intra- and inter-assay coefficients of variation were below 4% and 7%, respectively. The sensitivity of the assay was 2 mg/dl and the normal range < 30 mg/dl. Total cholesterol, high-density lipoprotein-cholesterol (HDL-C) and triglycerides were measured by enzymatic methods with a Hitachi 911 (Boehringer, Mannheim, Germany). HDL-C was determined in the supernatant after precipitation with phosphotungstate/Mg^{++} (Boehringer); LDL-C was obtained according to the Friedewald formula.

Due to limited residual plasma availability, plasma IGF-I was measured only at baseline and after 2 months of treatment with a double antibody radioimmunoassay using immunohistochemicals and trace provided by Medgenix (Fleurus, Belgium). The sensitivity of the assay was 150 pg/ml; the intra-assay and interassay coefficients of variation were 6% and 7.5%, respectively. In order to avoid interference from binding proteins, plasma samples were treated with acid ethanol. The normal range for IGF-I in age-matched adult subjects is 100–350 ng/ml.

Since the distribution of Lp(a) was skewed with a long tail towards higher values, a square root transformation of Lp(a) values and a log transformation of IGF-I values were necessary to achieve normality. The validity of these transformations was assessed by residual plots. The difference in the baseline values and the difference in the change in Lp(a) after 1 month of intervention between groups were estimated by the two sample *t*-test. Repeated measures analysis of the 6 month intervention period was performed by ANOVA. Given the large variation in Lp(a) values among subjects, a characteristic feature of Lp(a) [5], the effect of tamoxifen over time was expressed as the within-subject variation. Data on IGF-I were expressed as the least square mean and the 95% confidence interval (CI) was adjusted for baseline levels. The effect of tamoxifen on the change in IGF-I was expressed as a percentage change in view of the log transformation required. The association between the changes in Lp(a) and LDL-C over 6 months and between Lp(a) and IGF-I after 2 months of treatment were investigated using the Pearson correlation coefficients and scatter plots. The repeated measures analysis was applied to assess the influence of baseline values on the change in Lp(a) induced by treatment over time. All results except those for IGF-I are reported as the mean ± S.D.

RESULTS

The baseline characteristics of the 68 caucasian women are reported in Table 1. No significant difference was observed between the groups. The time course of serum Lp(a) levels during intervention is reported in Table 2. At baseline, there was no significant difference in Lp(a) between the groups. From baseline to 1 month there was a 23% (± 1.1%) reduction in mean Lp(a) in the tamoxifen group and an increase of 6% (± 1.1%) in the placebo group ($P = 0.033$ for the between-group difference). These figures were not obtained from the data shown in Table 2 but were based on the 30 women in the tamoxifen group and the 33 women in the placebo group with measurements of Lp(a) at both time points. No further variation in Lp(a) levels was observed up to the sixth month. The within-subject difference in Lp(a) levels between the groups was highly significant ($P = 0.00046$).

The change in Lp(a) levels over the 6 months of tamoxifen treatment was significantly influenced by the baseline values, a greater reduction being observed in the subjects with the highest baseline levels. Specifically, the 10 subjects with abnormal baseline Lp(a) levels (i.e. above 30 mg/dl) had a mean baseline value of 56.2 ± 25.6 mg/dl and showed an average 30% reduction during tamoxifen treatment

Table 1. *Main subject characteristics at baseline (mean ± standard deviation)*

	Tamoxifen	Placebo
	($n = 31$)	($n = 37$)
Age	51.4 ± 4.5 years	52.1 ± 4.6 years
Body mass index	24.7 ± 4.5	24.0 ± 3.0
Smoking habit		
Current	7	8
Former	5	10
Ever	19	19
Time since hysterectomy	9.6 ± 5.5 years	10.7 ± 6.7 years
Menopausal status		
Pre	5	9
Post	26	28
Current HRT	1	3
LDL cholesterol	149 ± 32 mg/dl	160 ± 29 mg/dl
HDL cholesterol	52 ± 13 mg/dl	55 ± 13 mg/dl
Plasma IGF-I	147.3 ± 66.5 ng/ml	141.7 ± 57.9 mg/dl

HRT, hormone replacement therapy; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IGF-I, insulin-like growth factor-I.

Table 2. Time course of lipoprotein(a) levels (mg/dl)

	Months of intervention				
	0	1	2	4	6
Tamoxifen	25.9 ± 26.3 (n = 31)	20.0 ± 18.9 (n = 30)	20.2 ± 23.3 (n = 31)	20.0 ± 19.0 (n = 30)	19.7 ± 20.9 (n = 31)
Placebo	22.7 ± 15.1 (n = 35)	24.2 ± 18.9 (n = 35)	24.3 ± 19.0 (n = 37)	23.3 ± 17.8 (n = 36)	26.0 ± 19.3 (n = 37)

Data are expressed as the mean ± standard deviation. The within-subject variation between the two groups over 6 months was significant (P = 0.00046).

(Figure 1). Furthermore, there was no effect of time (P = 0.27), which confirms that these changes in Lp(a) occurred within the first month of treatment.

Tamofixen induced a mean reduction in IGF-I levels of 23.5% (95% CI, − 14.1– − 31.8), whilst in the placebo group the mean reduction was only − 0.1% (95% CI, − 10.9–12.1) (P < 0.01, data not shown). At baseline, there was no correlation between Lp(a) and IGF-I levels in all subjects (r = − 0.067). Likewise, there was no correlation between the 2 month change in Lp(a) and IGF-I both in the tamoxifen and the placebo arm (r = 0.188 and 0.059, respectively, Figure 2).

As expected, treatment with tamoxifen induced a significant decline in mean LDL-C levels as compared with placebo; the percentage reduction from baseline to month 2 was 18% in the tamoxifen group and 2% in the placebo group. No significant change in HDL-C was observed (data not shown). At baseline, Lp(a) and LDL-C levels were uncorrelated (r = − 0.08). In the placebo arm, the changes in Lp(a) from baseline to 6 months were moderately correlated to the changes in LDL-C (r = − 0.47), but not at 2 months (r = − 0.11), whilst no correlation was observed in the tamoxifen arm (r = 0.06 at 2 months and 0.08 at 6 months).

DISCUSSION

Recent results from the US prevention trial indicate that tamoxifen can approximately halve the incidence of breast cancer and decrease by 20% the occurrence of bone fractures in healthy women aged 35–70 years at increased risk of breast cancer [2]. Although women aged 50 years or older developed a 4-fold increase of early stage endometrial cancer and a 3-fold increase in pulmonary embolism during tamoxifen treatment, the trial has been stopped as the benefits far outweighed the risks. Whilst no apparent reduction of CHD by tamoxifen has been reported [2], this might be due to the short follow-up and the young cohort age. In this framework, our study lends support to the broad spectrum of tamoxifen preventive potential, providing evidence for a stable 30% reduction in women with abnormal levels of Lp(a), an independent risk factor for premature CHD [6–9]. As young women with Lp(a) levels > 30 mg/dl have a 5-fold increased risk of developing CHD compared with women with levels ≤ 30 mg/dl [9], treatment with tamoxifen may result in a substantial reduction of CHD events.

Our observation appears noteworthy given the scarcity of safe and efficacious agents in young women with high Lp(a)

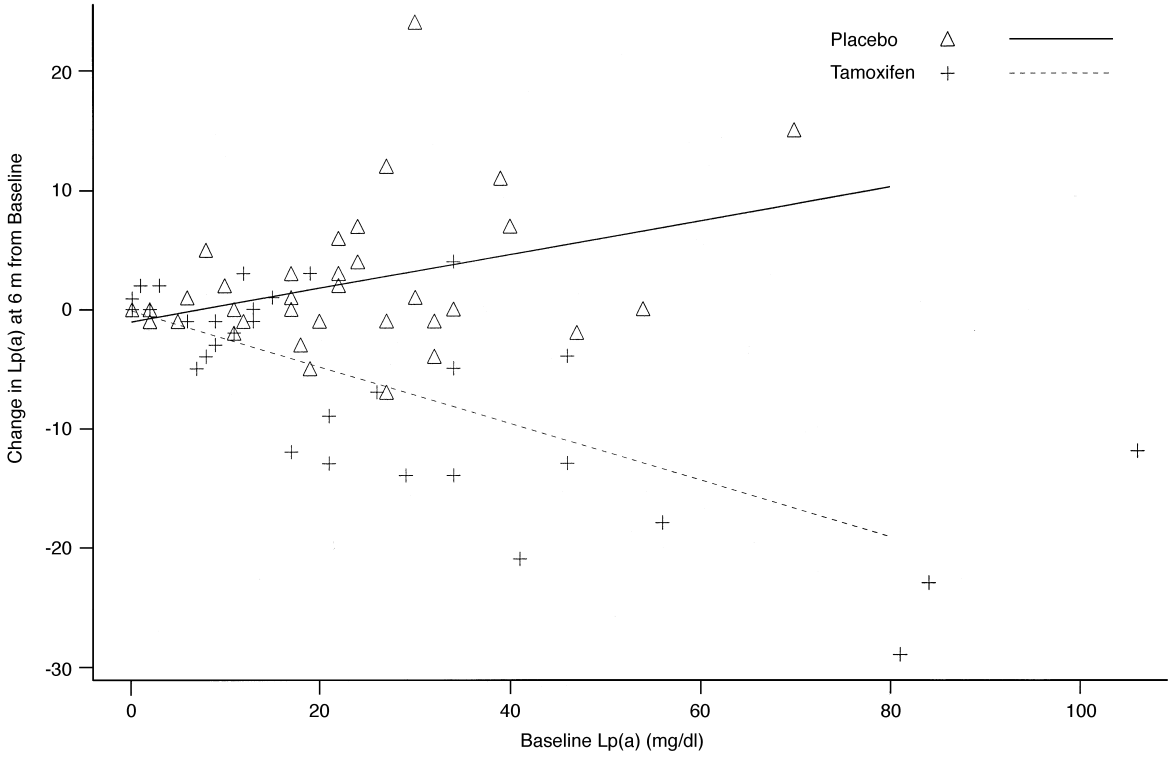

Figure 1. Effect of tamoxifen (+, _____) or placebo (△, – – – –) on the 6 month change in lipoprotein (a) (Lp(a)) levels according to baseline values. The interaction term between tamoxifen and baseline values on the change in Lp(a) levels is significant (P < 0.001).

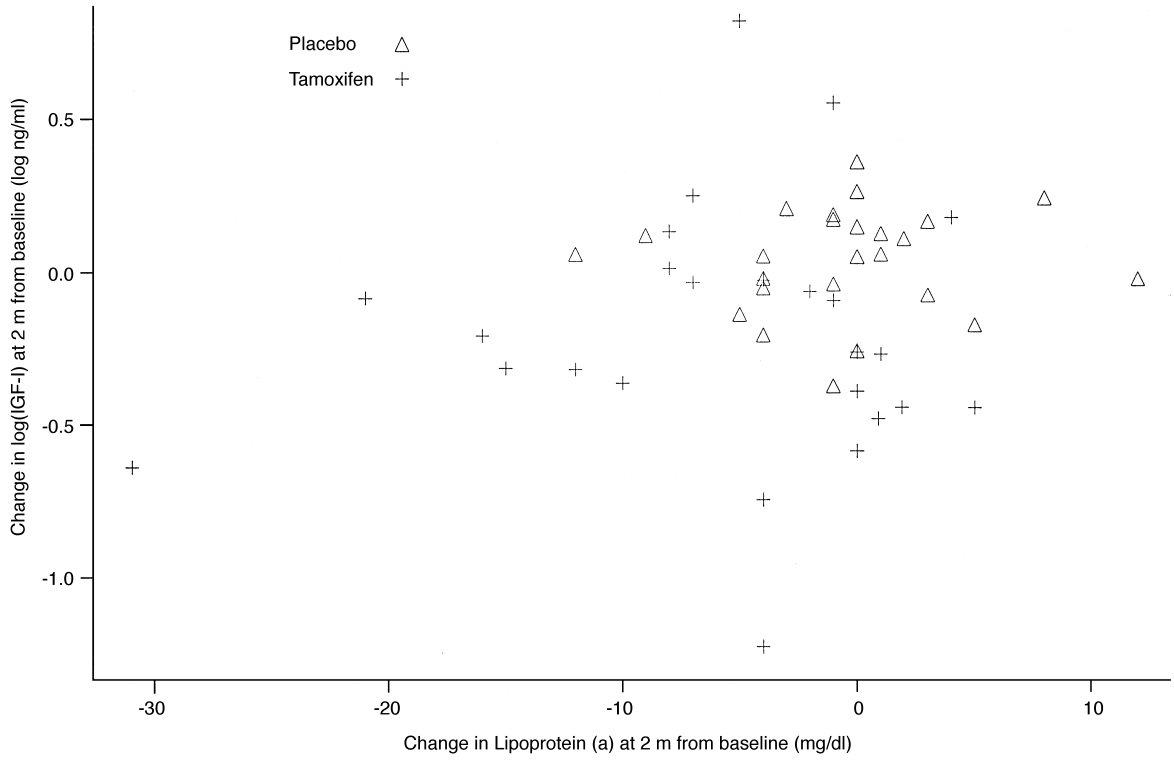

Figure 2. Plot of the lipoprotein (a) (Lp(a)) change upon insulin-like growth factor-I (IGF-I) change from baseline to 2 months of treatment (tamoxifen, $r = 0.188$; placebo, $r = 0.059$).

levels [5]. Indeed, the activity of niacin is attenuated by its significant toxicity [5], whilst hormone replacement therapy (HRT) is contra-indicated in women at increased risk for breast cancer. Moreover, there is evidence for a progressive loss of efficacy of HRT after an initial reduction in Lp(a) [11]. Whilst the risk/benefit ratio of tamoxifen administration must be carefully weighed, an increased risk of endometrial cancer or venous thromboembolic events appears to be restricted to postmenopausal women, consistent with the complex hormonal regulation of the pharmacological activity of tamoxifen [2]. Moreover, studies aimed at minimising the endometrial effect of tamoxifen with the concomitant use of HRT [14] or with a lower-dose [15] are currently underway. Thus, tamoxifen may qualify as a suitable preventive treatment for young women at-risk of the most prominent diseases of middle age, namely breast cancer and premature CHD.

Interestingly, the responses of Lp(a) and LDL-C to tamoxifen were not correlated, thus suggesting an independent pathway of tamoxifen modulation despite the similarity between the two lipoproteins. In this regard, it has recently been shown in transgenic mice that tamoxifen and oestrogen lower circulating apo(a) levels by suppression of apo(a) mRNA at the hepatic level [16]. Likewise, we did not observe any correlation between Lp(a) and IGF-I changes. This finding is in contrast with a previous observation in healthy subjects [11], but it is consistent with recent studies in subjects with familial hypercholesterolaemia treated with growth hormone (GH) [17] or subjects treated with IGF-I for Laron syndrome [18]. Whilst the discrepancy between our results and those previously reported [11] may be due to differences in sample size and population types, our results suggest that the modulation of Lp(a) and IGF-I by tamoxifen acts through independent pathways.

The effect of tamoxifen on Lp(a) strengthens the contention that, in addition to a major genetic influence, Lp(a) is under hormonal control. For example, GH increases while IGF-I and insulin decrease Lp(a) synthesis [19, 20]. Moreover, most ligands of the steroid/thyroid/retinoid receptor superfamily, particularly oestrogen [11], regulate Lp(a) levels. Whilst the physiological role of Lp(a) remains rather obscure and is confounded by the striking interindividual variations in serum concentration, current evidence indicates that this protein is involved in fibrinolysis inhibition, wound healing and growth regulation of smooth muscle arterial cells [5, 21].

In summary, we have shown that tamoxifen lowers Lp(a) and IGF-I by approximately 25% in healthy women, adding further support to the preventive potential of this compound.

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