REPORTS

Effect of Tamoxifen on Serum Insulinlike Growth Factor I Levels in Stage I Breast Cancer Patients

Michael Pollak, Joseph Costantino, Constantin Polychronakos, Sue-Ann Blauer, Harvey Guyda, Carol Redmond, Bernard Fisher, Richard Margolese*

Insulinlike growth factor I (IGF-I) has been shown to be a potent mitogen for breast cancer cells in vitro, and IGF-I receptors have been demonstrated on human primary breast neoplasms. In a randomized, placebo-controlled study, we document that administration of the antiestrogen tamoxifen to patients with breast cancer was associated with a statistically significant (P = .002) reduction in the serum level of IGF-I. The mean IGF-I level was 1.4 U/mL in the placebo-treated group and 0.9 U/mL in the tamoxifen-treated group. Because serum IGF-I level is growth hormone (GH) dependent and because data suggest that the pubertal surge in GH and IGF-I levels is sex steroid dependent, we speculate that the mechanism underlying our observation may involve blockade by tamoxifen of estrogen action in the hypothalamic-pituitary axis. We conclude that tamoxifen treatment reduces IGF-I levels and that this reduction may contribute to the therapeutic effect of the drug. [J Natl Cancer Inst 82:1693–1697, 1990]

Insulinlike growth factor I (IGF-I), also referred to as somatomedin C, is a 70-amino acid mitogen that stimulates cell proliferation in vitro and in vivo (*1*). The IGF-I receptor has been characterized and is a member of the tyrosine kinase family of mitogen receptors (*2*). Both estrogen receptor-positive and estrogen receptor-negative breast cancer cell lines are responsive to IGF-I (*3–7*); under some assay conditions, IGF-I appears to be a more potent mitogen than estradiol (*3*). IGF-I may have a physiologic role in lactation (*8*). Biochemical techniques have been used to demonstrate that human primary breast cancer cells exhibit IGF-I receptors (*9–11*), and immunohistochemical studies have localized IGF-I receptors to ductal epithelia in normal breast and neoplastic (nonstromal) components of human primary breast cancers (*12*). However, breast cancer cells appear not to produce IGF-I by an autocrine mechanism (*13*). Therefore, IGF-I from endocrine or paracrine sources may stimulate proliferation of breast cancer. Consistent with this view are studies showing inhibition of in vivo (*14*) and in vitro (*3–5*) proliferation of breast cancer by an antibody that blocks IGF-I receptors.

Circulating IGF-I levels are dependent on growth hormone (GH), and it has been demonstrated that IGF-I synthesis by hepatocytes is stimulated by GH (*15*). Recent reports have shown that there are also widespread extrahepatic sites of IGF-I production (*16,17*), but factors regulating IGF-I gene expression in nonhepatic tissues are less well characterized. Because specific IGF-binding proteins are present in serum, the vast majority of circulating IGF-I is present in a bound rather than a free state. Standard assays measure total (bound and unbound) IGF-I in serum.

The relationships between sex steroids, GH secretion, and IGF-I are complex and incompletely described. Sex steroids appear to have at least a permissive role for pituitary GH output, which is a key determinant of IGF-I levels (*18–22*). Both stimulatory (*23,24*) and inhibitory (*25*) direct influences of estrogens on IGF-I production have been described.

Tamoxifen is a competitive antagonist to the estrogen receptor and is commonly used in the adjuvant and palliative treatment of breast cancer. The site of antineoplastic activity of this drug has generally been regarded to be the estrogen receptor of neoplastic cells. In view of reports that both GH secretion and circulating IGF-I levels are dependent on sex steroids (*18–22*), we postulated that tamoxifen may act to block central nervous system and/or pituitary estrogen receptors (*26*), thereby reducing pituitary GH output and circulating IGF-I levels. To test this hypothesis, we measured serum IGF-I levels in patients participating in a clinical trial of tamoxifen versus placebo for treatment of early breast cancer.

Methods

IGF-I levels were measured in serum samples obtained at 9 AM from 69 patients. These patients had received for at least 3 months either tamoxifen or placebo as adjuvant treatment of stage I breast cancer according to the B14 pro-

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tocol of the National Surgical Adjuvant Breast and Bowel Project (NSABP) (*27*) at the Jewish General Hospital, Montreal.

A nonequilibrium double-antibody radioimmunoassay, employing recombinant human IGF-I (Amgen, Thousand Oaks, Calif) and an anti-IGF-I antibody provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, Md) was used (*28,29*). The reference serum standard used was from Ciba Corning Diagnostics (Irvine, Calif) and had a value of 228 ± 38 ng/mL (mean ± SD; n = 10) in terms of synthetic IGF-I. Although there has been concern over the possibility that IGF-binding proteins may interfere with the radioimmunoassay of IGF-I (*30*), the potential for interference appears to depend on the quantity of IGF-I-binding protein present and on the assay methodology. In the case of human serum at the dilutions that we use in our nonequilibrium assay, we (*29*) and others (*28,31*) have shown an absence of reactivity of binding protein purified by the method of Daughaday et al. (*30*). Under our assay conditions, we (*29*) and others (*28,31*) have demonstrated that unextracted sera give the same values as samples prepurified by acid Sephadex chromatography.

Investigators who carried out the assays were blinded as to patient treatment. They submitted patient-identifying numbers and IGF-I levels for analysis by biostatisticians who were aware of the treatment assignments.

No patient showed metastatic disease. Inasmuch as an interim analysis of the entire B14 protocol population carried out in 1987 had shown an advantage in relapse-free survival for patients in the tamoxifen treatment group, all subsequent patients received tamoxifen. Therefore, our random sample of patients in the B14 protocol comprises 48 tamoxifen-treated patients, but only 21 placebo-treated patients. IGF-I levels and other parameters in patients randomly allocated to receive tamoxifen before 1988 did not differ from those of patients registered to receive tamoxifen after 1988.

Differences in the mean values of parameters between patients in the tamoxifen and the placebo treatment groups were evaluated statistically using a t-test. We assessed possible relationships between IGF-I levels and parameters other than treatment by computing pairwise correlation coefficients and by inspecting stratified data. We performed stepwise multiple regression modeling using IGF-I concentration as the dependent variable to evaluate the relationship between IGF-I levels and tamoxifen treatment, relative to other possible relationships that might exist between IGF-I levels and age or body mass. To preserve the assumption of normality, we used the log of IGF-I concentrations in this regression modeling.

Table 1. Patient characteristics by treatment group

Characteristic	Treatment group		P
	Tamoxifen	Placebo	
No. of patients	48	21	—
% postmenopausal	75.0	61.9	.27
Mean age (yr)	56.7	52.9	.14
Mean wt (kg)	69.0	64.4	.12
Mean body mass index (kg/m³)	26.8	25.6	.42
Mean estrogen receptor levels (fmol/dL)	109.7	122.9	.58
Mean progesterone receptor levels (fmol/dL)	205.7	240.0	.67
Mean time on study (mo)	27.0	39.6	.003
Mean IGF-I levels (U/mL)	0.9	1.4	.002

Results

Table 1 lists selected patient characteristics, including mean weight and mean body mass index, which have been related to IGF-I levels in prior studies (*32*). As expected with the randomized study design, the pretreatment variables did not differ significantly between groups with the exception of time since surgery, which was anticipated due to the fact that all patients entered after the interim analysis of 1987 received tamoxifen.

The IGF-I level (mean ± SE) was 1.4 ± 0.15 U/mL for the placebo treatment group and 0.9 ± 0.07 U/mL for the tamoxifen treatment group. This difference is highly significant (P = .002). Figure 1 summarizes the differences between groups with respect to the distribution of IGF-I levels. These differences were marked at both extremes of IGF-I levels. While less than 5% of placebo-treated patients had levels lower than 0.5 U/mL, 16.7% of tamoxifen-

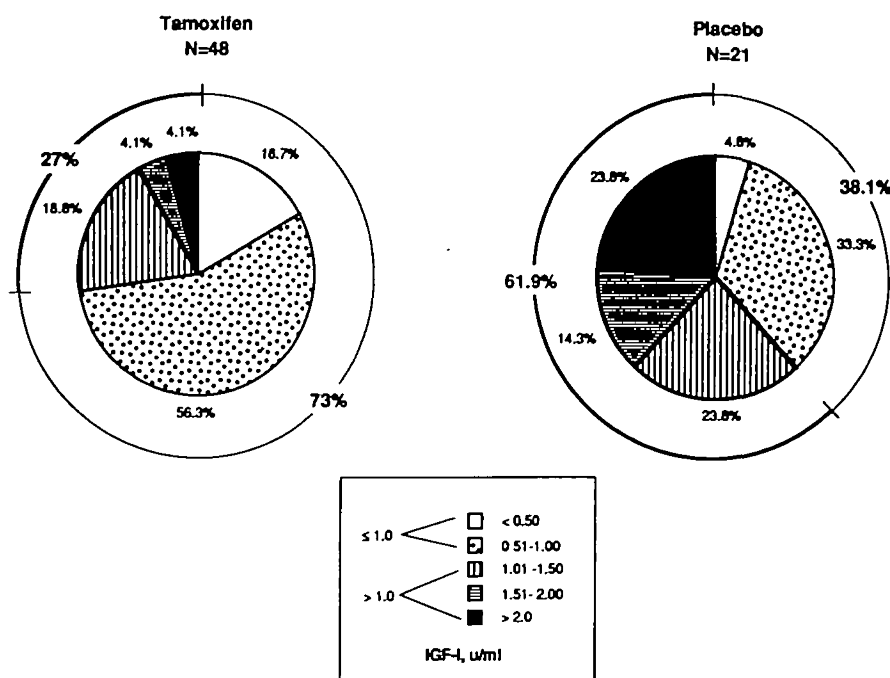

Figure 1. Distribution of serum IGF-I levels among patients with stage I breast cancer receiving tamoxifen (left) or placebo (right).

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Table 2. Mean IGF-I levels by treatment and age group

Age group (yr)	Tamoxifen-treated patients		Placebo-treated patients	
	No. of patients	Mean IGF-I levels (U/mL)	No. of patients	Mean IGF-I levels (U/mL)
≤50	12	1.06	8	1.48
51–60	12	1.00	9	1.50
≥61	24	0.82	4	0.97
Total	48	0.92	21	1.39

treated patients had levels in this range. Similarly, less than 5% of tamoxifen-treated patients had levels over 2.0 U/mL, whereas almost a quarter of the control group did.

Data presented in table 2 demonstrate a trend toward a decline in the level of IGF-I with age. This trend is consistent with the findings of previous studies (*33*). For each age group, a reduction in the level of IGF-I was associated with tamoxifen usage. Although extremes of diet have previously been noted to affect IGF-I levels (*32*), we did not observe any correlation between weight, percent ideal body weight, or body mass index and IGF-I level in this group of patients (data not shown).

To determine if the difference in IGF-I levels persists after adjustment for age and time on study, we performed stepwise multiple regression modeling using log IGF-I as the dependent variable. The results of this modeling showed that tamoxifen usage was a significant determinant of IGF-I level, even after adjustment for these variables, and that only tamoxifen usage and age were significant variables predicting IGF-I levels (table 3).

Discussion

Tamoxifen is one of the most effective antineoplastics in use today and is prescribed to millions of women worldwide for the adjuvant or palliative treatment of breast cancer. In this randomized, placebo-controlled study, we demonstrated that use of this drug was associated with a statistically significant reduction in the level of serum IGF-I. Our study was carried out in patients with stage I estrogen receptor-positive breast cancer, but there is no reason to suppose that the effect is confined to this subgroup of tamoxifen users.

Table 3. Stepwise multiple regression modeling of log IGF-I as dependent variable

Model	Variable	Regression coefficient	*P*
1	Age	−0.009	.002
	Treatment	−0.145	.020
	Time on study	−0.001	.456
2	Age	−0.009	.002
	Treatment	−0.129	.027

The fact that response to tamoxifen is correlated with estrogen receptor level of tumors has been regarded as strong evidence that the site of tamoxifen action is at the estrogen receptor of the neoplastic cell, where the drug acts as a competitive antagonist and blocks estrogen-stimulated proliferation. However, recent reports (*10,11*) demonstrate a strong positive correlation between estrogen receptor level and IGF-I receptor level in primary breast cancers. Therefore, it is possible that a class of estrogen receptor-positive, IGF-I receptor-positive breast cancers exists that has the potential to respond to both direct and indirect (IGF-I-lowering) effects of tamoxifen.

Laboratory studies (*3–7,14*) suggest that at least a subset of breast cancers is IGF-I responsive. These results are consistent with the possibility that the IGF-I-lowering effect of tamoxifen is related to its therapeutic activity. However, in vitro dose-response curves relating concentration of IGF-I to stimulation of proliferation of breast cancer cells cannot be directly extrapolated to in vivo conditions for a number of reasons, including differences in concentrations of IGF-binding proteins. Therefore, further work is required to determine if the observed decline in serum IGF-I level associated with tamoxifen usage is of sufficient magnitude to reduce proliferation of breast cancer cells.

Because the patients in this study were on an adjuvant-treatment protocol and had no recurrent disease, we cannot determine at this time if clinical response to tamoxifen is correlated with a sustained decline in IGF-I. Current models to explain the clinically important phenomenon of development of tamoxifen resistance following an initial response to the drug emphasize tumor-related factors such as selection for estrogen receptor-negative clones during neoplastic progression. The data presented here suggest that host-related factors may also play a role, in that relapse following initially successful treatment may be associated with escape from suppression of IGF-I levels. Further studies examining serum IGF-I levels prior to and during tamoxifen therapy for metastatic disease will be useful in addressing this issue.

The hypothesis that individuals with relatively high levels of IGF-I have an increased risk of developing breast cancer and/or a poorer prognosis if they develop the disease remains speculative at this time. However, in the context of data suggesting less aggressive metastatic behavior of breast cancer in older women (*34*), it is of interest that we (table 2) and others (*33*) have observed that IGF-I levels decline with age. While many host and tumor factors determine biologic behavior of neoplasms, the hormonal milieu of older women, including their relatively low levels of IGF-I, may be an important factor here.

A nonrandomized retrospective survey (*35*) of IGF-I levels in patients with breast cancer reported while our study was in progress is difficult to interpret with respect to the effect of tamoxifen because the sample was small and the study group was heterogeneous with respect to treatment and breast cancer stage. However, the results from this survey are consistent with our findings.

The mechanism by which tamoxifen lowers IGF-I levels remains unclear. In view of reports indicating that sex steroids play a role in regulating pituitary GH secretion, we propose that one mechanism by which tamoxifen (and presumably oophorectomy) lowers IGF-I levels involves a suppression of GH secretion by the pituitary gland. It is of interest that a biphasic response of IGF-I levels to estrogen administration has been described (*18*), with physiologic levels increasing IGF-I, but pharmacologic levels reducing IGF-I. This result implies that the mechanism of action of high-dose estrogens in breast cancer therapy may also involve IGF-I suppression. Another possible mechanism of action of tamoxifen involves attenuation of a direct stimulatory effect of estrogen on IGF-I gene transcription (*23,24*). Finally, our data are consistent with the possibility that the reduction in serum IGF-I associated with tamoxifen usage is

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secondary to a tamoxifen-induced decline in the levels of various serum IGF-I-binding proteins that may be influenced by estrogen and/or GH levels (*36,37*). The observed reduction in total serum IGF-I may or may not be associated with a change in bioactive, free IGF-I at IGF-I-responsive tissues, and further studies to address this issue are justified.

In view of the pulsatile nature of GH secretion, assay of single serum samples does not accurately estimate 24-hour GH output. To test the hypothesis that the mechanism underlying the observed decrease in IGF-I levels involves a reduction in pituitary GH output, prospective studies involving provocative testing and 12-hour GH profiles are planned. Experiments with cultured sheep somatotropes support this hypothesis, as in this system tamoxifen suppresses basal and GH-releasing factor-stimulated GH release (Malaab SA, Pollak MN, Goodyer CG: manuscript submitted for publication).

The concept that tamoxifen acts as an antineoplastic simply by acting as a competitive antagonist at the estrogen receptor of neoplastic cells appears to be incomplete. Tissue culture studies indicate that at the level of the neoplastic cell, the drug acts locally in a complex manner that involves not only estrogen receptor blockade, but also alterations in autocrine production of both stimulatory and inhibitory growth factors (*38*). Our data indicate that tamoxifen also has systemic actions that may contribute to its therapeutic effect. The relative importance of the systemic and local actions of tamoxifen with respect to the therapeutic effect of the drug in vivo requires further study.

Data concerning the IGF-I responsiveness of breast cancers and the IGF-I-lowering effects of tamoxifen may lead to the development of new forms of hormonal therapy more potent than those currently in use. For example, more complete suppression of IGF-I levels might be achieved by combining IGF-I-lowering drugs such as somatostatin analogues (*39*) or GH-releasing hormone antagonists (*40*) with tamoxifen, to achieve a "selective medical hypophysectomy." If tamoxifen lowers IGF-I levels by suppressing GH (itself a lactogenic hormone), it may be synergistic with the prolactin-lowering measures that have been shown to reduce the proliferation of human breast cancer cells (*41*). Competitive antagonists to lactogenic hormone receptors (*42*) and the IGF-I receptor, currently under development, may contribute to pharmacologic manipulation of IGF-I.

Our findings may also have relevance to efforts to improve the effectiveness of hormonal treatments for neoplasms other than estrogen receptor-positive breast cancer (*43–48*) and to the use of tamoxifen as a chemopreventative agent (*49,50*).

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Prediction of Postoperative Clinical Course by Autologous Tumor-Killing Activity in Lung Cancer Patients

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Fifty patients with primary localized lung cancer were tested at the time of surgery for the ability of their lymphocytes to kill autologous, freshly isolated tumor cells, and the assay was evaluated for prognostic significance. Peripheral blood lymphocytes of 27 patients (54%) demonstrated significant autologous tumor-killing activity in 6-hour ^{51}Cr-release assays. Twenty-three of the 27 patients with autologous tumor-killing activity remained tumor free and survived more than 5 years after curative surgery, while all 23 who were negative for autologous tumor-killing activity relapsed by 18 months after surgery and died within 42 months after surgery. The differences in survival curves for the two groups were highly significant ($P <$ .00003). Autologous tumor-killing activity was not correlated with natural killer (NK) cell activity against K562 human myeloid leukemia cells or proliferation of lymphocytes stimulated with autologous, freshly isolated tumor cells in mixed culture. There were no differences in total survival between patients with positive results and those with negative results in tests of NK cell activity and autologous mixed lymphocyte–tumor culture reaction. These results indicate that autologous tumor-killing activity is a meaningful prognostic indicator and provide evidence for immunological control of tumor growth and metastasis. According to our preliminary data, it is unlikely that lung cancer patients who remain tumor free after 60 months of follow-up will develop recurrence or die from the disease. We are conducting a study to determine whether induction of autologous tumor-killing activity before surgery, by treatment with biological response modifiers, can improve the clinical outcome in patients who do not naturally have this potential. **[J Natl Cancer Inst 82:1697-1701, 1990]**

Natural killer (NK) cells are implicated as important effectors against tumor because of their ability to kill tumor cells without prior sensitization and in the absence of major histocompatibility complex (*1,2*). It is, however, difficult to interpret the data on cytotoxicity against cultured tumor cell lines, since tumor cells alter their susceptibility to cell-mediated lysis when cultured in vitro (*3,4*). For a better evaluation of cytotoxicity of lymphocytes against tumor cells in cancer patients, autologous combinations of fresh effector and target cells have been used. In these studies, peripheral blood lymphocytes from 10%–50% of cancer patients, depending on tumor types and metastases, expressed lysis of tumor cells freshly isolated from the same patients (*4–8*). The results obtained from our studies of cell populations and single cells indicate that when large granular lymphocytes obtained from the blood and tumor tissues of cancer patients are negative for CD3 and positive for CD16 cell-surface antigens, they lyse autologous, freshly isolated tumor cells (*9,10*) and release a novel cytotoxic factor with lytic effects on autologous and allogeneic fresh human tumor cells (*11*). In addition, results of a previous study showed that autologous tumor killing was also mediated by lymphocytes that were positive for CD3 and negative for CD16; this mediation was performed by T lymphocytes in patients with localized neoplasms and primarily by large granular lymphocytes in patients with metastatic tumors (*4*).

Autologous mixed lymphocyte–tumor culture reaction has been used to investigate the recognition of autologous tumor cells by autologous lymphocytes

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