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Papers

Suppression of Serum Insulin-like Growth Factor-1 Levels in Breast Cancer Patients during Adjuvant Tamoxifen Therapy

Andreas Friedl, V. Craig Jordan and Michael Pollak

Serial IGF-1 levels in patients prior to and during adjuvant tamoxifen (TAM) treatment were followed in a retrospective study. Serum IGF-1 levels were determined by radioimmunoassay in 19 patients taking TAM and 19 controls, matched for age, body weight and other treatments. IGF-1 levels at 2 years were significantly lower in TAM patients ($P \leq 0.05$) compared to control patients. We observed a significant mean drop from pre-treatment to treatment IGF-1 levels by 19.9% in the TAM group ($P \leq 0.005$), but also noted a mean 11.4% decline in the control group ($P \leq 0.025$). A subgroup analysis suggested that premenopausal were relatively resistant to the IGF-1 lowering effects of TAM as compared to postmenopausal women.

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INTRODUCTION

RESPONSE to tamoxifen (TAM) in advanced breast cancer [1] and prolongation of disease-free survival in the adjuvant setting [2, 3] is largely restricted to patients with oestrogen receptor (ER)-positive primary tumours. However, a minority of approximately 13% of patients with ER-negative tumours also respond to TAM [1]. Additionally there are some breast cancer patients who experience a benefit from TAM after they have failed ablative hormonal treatment [4] or other endocrine manipulation [5]. The recently published overview analysis by the "Early

Breast Cancer Trialists Collaborative Group", which includes 30 000 breast cancer patients treated with TAM provides the strongest evidence in favour of a TAM effect on ER-negative tumours [6]. The report concludes that TAM reduces the incidence of recurrences in the "ER-poor" subgroup (as defined by ER negative or < 10 fmol/mg protein) by 13%. An 11% reduction in mortality is seen in the same subgroup. Both results are statistically significant.

It appears that the classical concept of antioestrogen action, which is based on competitive inhibition of oestrogen binding to

the ER, incompletely describes the clinical situation. Alternate mechanisms of antioestrogen action independent of the ER have been suggested, including inhibition of protein kinase C (PKC) [7], binding to calmodulin [8], association with "antioestrogen binding sites" (AEBS) [9], immunomodulation [10] and most recently, stromal induction of transforming growth factor β_1 [11]. Any or all of these mechanisms could play a role in controlling ER-negative disease.

More recently it has been shown that polypeptide growth factors, acting in a paracrine or endocrine fashion, could provide an additional pathway for indirect oestrogen and antioestrogen action [12]. Insulin-like growth factor (IGF)-1 is an interesting candidate in this respect for several reasons: it acts as a strong mitogen for breast cancer cells [13], and type 1 insulin-like growth factors receptors, which mediate the mitogenic effect of IGF-1, have been found to be almost ubiquitously present on breast cancer cell lines and biopsy material [13, 14]. Serum IGF-1 originates primarily from synthesis in the liver. Here, IGF-1 production is under positive growth hormone (GH) control and acts as an endocrine second messenger. *In situ* hybridization experiments demonstrated that IGF-1 mRNA is also present in stromal cells of breast cancer tissue, but not the neoplastic cells themselves [15], thus making a case for a function as paracrine growth signal. Suppression of IGF-1 production could provide a novel approach for breast cancer therapy. Somatostatin has been evaluated and shown to lower IGF-1 levels and it is possible that the pharmacological administration of somatostatin could have value as a breast cancer therapy. Interestingly, recent studies have shown that IGF-1 levels are lower in breast cancer patients treated with tamoxifen than in control patients [16, 17].

In this article we extend these previous reports by measuring pretreatment values in all patients in addition to IGF-1 levels during treatment. This individual follow-up information is valuable since IGF-1 is known to display a wide person-to-person variability. The second (treatment) serum sample in our study was obtained after 2 years of TAM therapy; in a limited number of patients IGF-1 levels were assayed for follow-up periods of 5 years. The rationale was to determine changes in IGF-1 during long-term TAM therapy, a therapeutic approach that is considered to be standard practice today [18].

PATIENTS AND METHODS

Patients

Patients who had undergone mastectomy or lumpectomy for stage I, II or III breast cancer between August 1977 and May 1986 and who were followed at the Wisconsin Comprehensive Cancer Center were studied retrospectively.

We investigated two sets of patients. The first set consisted of 19 patients on long-term TAM therapy (10 mg twice a day). 14 of them had received 4–19 cycles of adjuvant chemotherapy in addition to endocrine therapy. 19 control patients, who had

never received TAM and who matched the TAM patients according to age (± 2 years), approximate body weight and treatment other than TAM were assigned to these 19 TAM patients. Patients' characteristics are summarised in Table 1. 12 of these 19 TAM patients were premenopausal at the time of diagnosis, 7 were postmenopausal. The second set of patients consisted of 18 long-term TAM patients. The mean age in this second population was 56 years. 15 of them had received adjuvant chemotherapy in addition to TAM. All patients studied were free of recurrence during the observed interval.

Serum samples

At least two serum samples were assayed in all patients. The first one had been obtained prior to the initiation of any adjuvant therapy or the observation interval in some control patients. In those TAM patients who had a matched paired control and in their assigned control patients, the second sample was obtained 2 years after the initiation of adjuvant therapy. At that timepoint all TAM patients were still on TAM and those patients who had received chemotherapy had long completed their regimen. In the remaining TAM patients (without controls), the second sample had been obtained at 6–12 months. All samples had been stored at -70°C at the serum bank of the Wisconsin Comprehensive Cancer Center in Madison.

IGF-1 assay

IGF-1 was measured as described previously [17]. Briefly, a radioimmunoassay was performed on diluted serum, following acid-ethanol precipitation. The anti-IGF-1 antibody was provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, Maryland). All samples from individual patients and their controls were assayed in one run.

Statistical analyses

Appropriate data sets (pretreatment vs. treatment, TAM vs. control) were compared, using the Student's *t*-test for paired variables. The age versus IGF-1 level relationship was examined by linear regression analysis. The significance of the correlation was determined by the F-test.

RESULTS

Figure 1 depicts pretreatment and treatment IGF-1 levels of 19 TAM patients and their respective controls. The values of individual patients are connected by lines. In Table 2 the results are summarised in numerical form as means \pm standard error (S.E.).

There was no significant difference in pretreatment IGF-1 values between TAM and control patients. IGF-1 levels during treatment are significantly lower in the TAM group compared to the control group ($P \leq 0.05$). Comparing pretreatment with

Table 1. Characteristics of 19 TAM patients and their paired, matched controls

	TAM	Control
Age [mean (min; max)]	49(34; 64)	49(34; 62)
Weight [mean \pm S.D.]	64.2 \pm 9.16	64.3 \pm 10.5
Therapy: Chemotherapy + TAM	14	—
Chemotherapy	—	14
TAM	5	—
Observation	—	5

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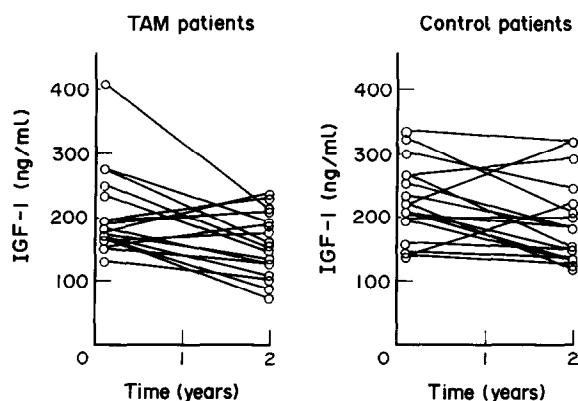


Fig. 1. IGF-1 levels in 19 TAM patients and 19 control patients before initiation of adjuvant therapy (or observation) and 2 years later. Values of individual patients are connected by a line.

treatment values, there is a significant mean decrease of 19.9% ($\pm 6.4\%$ S.E.) in the TAM group ($P \leq 0.005$), but also to a lesser degree ($11.4\% \pm 6.5\%$ S.E.) in the control group ($P \leq 0.025$). In the group of 18 TAM patients without matched controls we observed a mean 28.6% ($\pm 8.5\%$ S.E.) decline in IGF-1 levels from $125.7 (\pm 12.7$ S.E.) to $81.4 (\pm 8.8$ S.E.) ng/ml that was significant at a P -value of less than 0.05. When all 37 TAM patients were analysed together, the drop was 24.1% ($\pm 5.3\%$ S.E.) and reached a significance level of $P \leq 0.0005$. However, the difference in declines of IGF-1 level between the TAM patients and the control group failed to reach statistical significance.

In a subgroup analysis the control patients (who had never received TAM) were stratified according to their postoperative management. The 14 patients who had received chemotherapy on average experienced a 7.4% ($\pm 8.25\%$ S.E.) decrease from $218.4 (\pm 16.2$ S.E.) to $196.5 (\pm 18.5$ S.E.) ng/ml as compared to a 22.5% ($\pm 7.1\%$ S.E.) decline from $213.0 (\pm 30.9$ S.E.) to $160.4 (\pm 22.0$ S.E.) ng/ml in the 5 observation patients. The equivalent analysis in the TAM patients revealed a mean 14.4% ($\pm 7.4\%$ S.E.) drop in the TAM plus chemotherapy group from $208.5 (\pm 20.3$ S.E.) to $167.8 (\pm 12.1$ S.E.) ng/ml versus a mean 35% ($\pm 11.7\%$ S.E.) drop in the TAM alone group from $201.0 (\pm 17.8$ S.E.) to $131.5 (\pm 24.8$ S.E.).

In another subgroup analysis we stratified the patients according to their menopausal status at the time of diagnosis. The 7 postmenopausal TAM patients experienced a mean 34.1% ($\pm 8.5\%$ S.E.) drop in IGF-1 levels, as compared with a mean 7.11% ($\pm 9.9\%$ S.E.) decline in their respective control patients. These results were statistically different ($P \leq 0.05$). In the 12 premenopausal TAM patients and their controls we observed a mean 11.5% ($\pm 8.2\%$ S.E.) and 13.9% ($\pm 8.7\%$ S.E.) decline, respectively. The drops in the premenopausal patients were not

Table 2. IGF-1 serum values expressed as means \pm standard error

	IGF-1 (ng/ml) pre-treatment	IGF-1 (ng/ml) 2 years	Decline (%)	Significance
TAM	206.5 ± 15.4	158.2 ± 11.3	19.9 ± 6.4	$P \leq 0.005$
Control	217.0 ± 14.0	187.0 ± 15.0	11.4 ± 6.5	$P \leq 0.025$
Significance	n.s.	$P \leq 0.05$	n.s.	

n.s. = not statistically significant.

Table 3. Subgroup analysis of IGF-1 values according to menopausal status

	TAM	Control	Significance
Premenopausal			
IGF pretreatment (ng/ml)	194.8 ± 12.8	211.8 ± 16.5	n.s.
IGF treatment (ng/ml)	165.8 ± 12.3	174.0 ± 14.7	n.s.
n	12	12	
Decline (%)	11.5 ± 8.2	13.9 ± 8.7	n.s.
Postmenopausal			
IGF pretreatment (ng/ml)	226.6 ± 36.3	225.9 ± 26.8	n.s.
IGF treatment (ng/ml)	145.3 ± 22.9	209.2 ± 31.8	$P \leq 0.05$
n	7	7	
Decline (%)	34.1 ± 8.5	7.1 ± 9.9	$P \leq 0.05$

n.s. = not significant.

significantly different. The results of this subgroup analysis are summarised in Table 3.

Pretreatment values of all patients were correlated with age. A linear regression analysis revealed decline of growth factor levels with age (Fig. 2). The correlation coefficient was only 0.39, but was found to be significantly different from zero by F -test.

In 5 patients, IGF-1 was measured in serum samples that had been collected over a 5-year period. Figure 3 demonstrates the heterogeneity of IGF-1 response to TAM treatment. While there is a pronounced and maintained suppression in patient "A", the growth factor level is essentially unaffected in patient "B".

DISCUSSION

IGF-1 is a mitogen for the majority of breast cancer cell lines [13]. In fact it appears to be among the most potent mitogenic polypeptide growth factors acting on breast cancer cells. Its central role in the regulation of proliferation is demonstrated by the observation that antibodies directed against the ligand-binding domain of the type-1 IGF receptor are capable of inhibiting growth of an ER-negative breast cancer cell line [19].

These observations, and the fact that IGF-1 levels can be manipulated by steroid hormones, have led investigators to study the effect of the non-steroidal antioestrogen tamoxifen on serum levels of this growth factor [16, 17]. Our results are in agreement with, and extend these previous publications. IGF-1

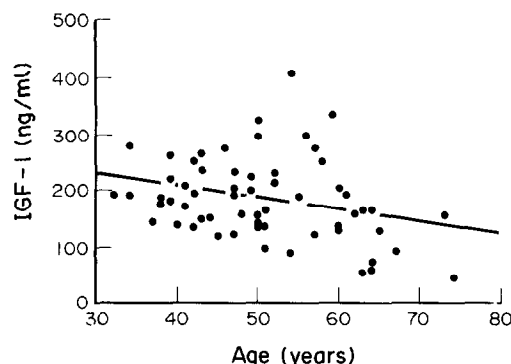


Fig. 2. Relationship between pretreatment (or preobservation) IGF-1 levels of all patients with age, regardless of subsequent therapy ($n = 57$). The correlation coefficient is only 0.3, but the decline of IGF-1 with advancing age is significant ($P < 0.025$).

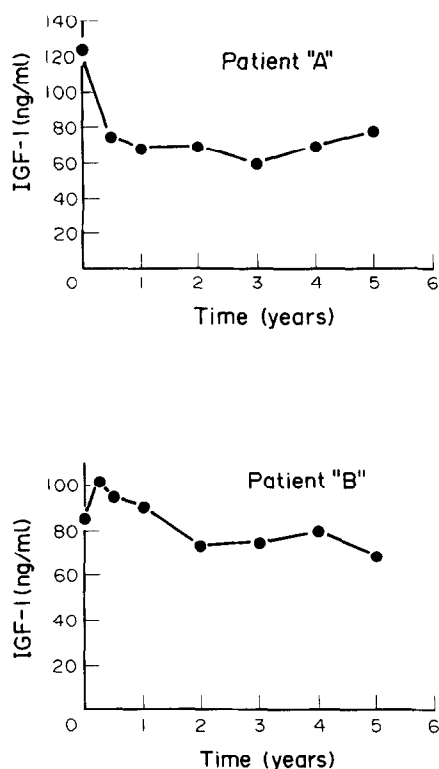


Fig. 3. Heterogeneity of IGF-1 suppression by TAM: while there is a profound suppression of IGF-1 levels in patient "A", which is maintained over 5 years, only a minimal effect is seen in patient "B".

levels are lower in patients during TAM therapy compared to controls. We also observed a highly significant decline of IGF-1 values after the initiation of TAM therapy.

This is, however, the first study that incorporates pretreatment and treatment samples of TAM and matched control patients. Surprisingly there was a significant decrease in IGF-1 levels from pretreatment to treatment in the control group. Mechanisms independent from TAM have to be considered to explain this observation. First, the decrease could simply be age-dependent. It is unlikely that advancing age is the only factor responsible for the IGF-1 drop observed in our control group, since, according to the regression analysis results of our data, we would only predict a decrease by 4.4 ng/ml over the observation period of 2 years. This contrasts with an actual mean drop of 30.0 ng/ml in the control group. Second, the lowering of IGF-1 values might be caused by adjuvant chemotherapy which was received by 14 out of 19 control patients. A subgroup analysis revealed, however, that the decline was actually more pronounced in the group of patients who had not received chemotherapy (22.5% vs. 7.4%). The third possibility is that pretreatment IGF-1 levels were elevated above baseline, possibly due to stress secondary to surgery. The vast majority of pretreatment samples had been obtained after surgery. The decrease of IGF-1 levels in the control patients is presumably the reason why there was no significant difference in the decline of IGF-1 from pretreatment to treatment values, between TAM and controls, despite the fact that there was a significantly lower ($P \leq 0.05$) serum IGF-1 level in TAM-treated women compared with controls.

A subgroup analysis according to menopausal status revealed that postmenopausal TAM patients experienced a significant drop in IGF-1 levels compared to their controls, while the growth

factor levels remained virtually unchanged in the premenopausal group (see Table 3). TAM is known to raise oestradiol levels in premenopausal women and it is possible that this effect could counteract any potential suppression of IGF-1 by TAM in this group. This hypothesis could, however, be challenged by the clinical observation that 1 year after completion of chemotherapy the ovaries are usually not functional. Another possibility is that pituitary GH secretion and serum IGF-1 levels, which are known to decline with age, are more susceptible to inhibition in elderly individuals. The subgroup analysis according to menopausal status should be interpreted with caution, since it was not an objective of the original study and the patient population is small. However, it is an interesting observation that warrants further investigation. Interestingly we also observed a more dramatic mean decline of 28.6% in the group of 18 TAM patients without controls who had an average age of 56 years in comparison to a mean age of 49 years in the group of TAM patients with controls. Other investigators who studied patients significantly older than those in our population found TAM-induced IGF-1 suppression of up to 80.6% [20].

We included in our study patients who had received chemotherapy in addition to TAM and attempted to control for the effect of chemotherapy by assigning control patients who were matched according to therapy other than TAM. We are aware of the limitations of this study design and acknowledge that differences between the two groups can not necessarily be attributed to the effect of TAM alone. Drug interactions, for example, could also play a role.

The previously described age-dependent decline of IGF-1 levels [21] was confirmed by our study. This result emphasises the need for age-matched controls, when evaluating possible treatment effects on this polypeptide growth factor.

It is possible that the mechanism by which TAM could lower IGF-1 levels involves the hypothalamo-pituitary axis. Physiological concentrations of oestrogens may increase GH secretion by the pituitary gland [22, 23]. In the rat ER could be localised to GH-releasing factor producing neurons in the hypothalamus and GH cells in the anterior pituitary gland [24] which implies a function of oestrogens in the control of this hormone. More recently we were able to demonstrate that TAM administration decreases GH secretion in the rat [26] and in cultured lamb pituitary cells [27].

TAM could also exert its suppression effect on IGF-1 via a more direct route. There are numerous reports that oestrogens have a direct stimulatory effect on IGF-1 gene expression in various tissues, including pig uterus [28] and in an osteosarcoma cell line [29].

The most important question regarding the biological and clinical significance of our findings is whether a lowering of IGF-1 by up to 50% in some individuals (see Fig. 3, patient A) can have an inhibitory effect on tumour growth. An answer cannot be given with certainty because the pharmacokinetics of IGF-1 are not known. *In vivo*, a tumour response would depend on local tissue concentrations of this growth factor, abundance of type-1 IGF receptors, insulin receptors, and concentrations of the various IGF-binding proteins in serum and tissue. To complicate matters further, many of these factors are under the influence of steroid hormones, antioestrogens and insulin-like growth factors. A very recent study by Kiang and co-workers shows that the extent of TAM-induced IGF-1 suppression correlates with clinical response in patients with metastatic breast cancer [20]. These data suggest that lowering of IGF-1 could have clinical significance.

It has been shown that oestrogens and IGF-1 have a synergistic effect on the growth of MCF-7 breast cancer cells. Oestradiol is capable of up-regulating type-1 IGF receptors and hence sensitises these ER-positive cells to the actions of IGF-1 [30]. Conversely, TAM might exert its tumoricidal effect on hormone responsive cells not only by direct ER-mediated growth inhibition but also by down-regulating type 1 IGF receptors present on tumour cells and reducing levels of circulating IGF-1.

An interesting finding of our analysis is the heterogeneity of changes in IGF-1 levels within the studied patient population. While some patients show a pronounced and sustained IGF-1 suppression (exemplified by patient "A" in Fig. 3), IGF-1 levels appear to be virtually unaffected in others. It will be of interest to determine if serum IGF-1 represents a "host-related" as distinct from "tumour-related" prognostic factor, and if decline in serum IGF-1 is correlated with response to TAM treatment.

Recent clinical evidence convincingly further supports the benefit of prolonged adjuvant endocrine therapy, making the indefinite administration of TAM a therapeutic option. One important aspect of our study in contrast to previous reports is the incorporation of the concept of long-term TAM therapy. Treatment samples had been obtained 2 years after initiation of TAM therapy. In a small number of patients we were able to show that IGF-1 suppression is maintained for the duration of 5 years.

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