Tamoxifen reduces serum insulin-like growth factor I (IGF-I)

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Summary

Antiestrogens are widely used in the management of hormonally responsive breast cancer in both adjuvant and palliative settings, and are currently being evaluated as chemopreventive agents. The classical mechanism of action of these drugs involves inhibition of estrogen-stimulated neoplastic cell proliferation by blockade of estrogen receptors present on breast cancer cells. This paper reviews recent clinical and laboratory data that suggest that the commonly used antiestrogen tamoxifen also acts to reduce serum IGF-I levels. Estrogens appear to play a permissive role in growth hormone (GH) release by the pituitary gland and GH is known to stimulate IGF-I expression by hepatocytes. It is therefore possible that blockade of estrogen receptors in the hypothalamic-pituitary axis by tamoxifen interferes with GH release, leading to reduced hepatic IGF-I expression. In view of results suggesting that IGF-I is a more potent mitogen than estradiol for breast cancer cells and data demonstrating a positive correlation between estrogen receptor level and IGF-I receptor level of breast cancer cells, the IGF-I lowering effect of tamoxifen may contribute to the cytostatic activity of the drug. The interrelationships between steroid hormone physiology and IGF-I physiology may have relevance to a variety of commonly used treatments for hormonally responsive cancers.

Insulin-like growth factor I (IGF-I) responsiveness of breast cancer

A number of studies over the past decade provide support for the view that IGF-I [1] is an important mitogen for breast cancer, as well as certain other malignancies [2,3]. Studies of immortalized breast cancer cell lines in tissue culture demonstrate impressive stimulation of proliferation by IGF-I and inhibition of proliferation by blockade of type I IGF receptors [4-8]. In retrospect, the mitogenic responsiveness of breast cancer cells to pharmacological concentrations of insulin demonstrated in older studies [9] is also consistent with an effect mediated by the type I IGF-I receptor. The remarkable inhibition of *in vivo* breast cancer proliferation resulting from blockade of IGF-I receptors [10] provides strong evidence to support the hypothesis that at least certain breast cancers are IGF-I responsive. Involvement of IGF-I in mammary cancer should come as no surprise given the role of this peptide in lactogenesis [11].

Studies of human breast cancer tissue have demonstrated specific IGF-I binding sites [12-17],

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which have been localized by immunocytochemical studies to the neoplastic (as distinct from stromal) component of the tumour [18]. As there is a loose positive correlation between IGF-I receptor expression and estrogen receptor expression [13], it is likely that tumours that are identified by estrogen receptor assays as being hormonally responsive are influenced by both estrogens and by IGF-I. However, there also exists a class of estrogen receptor negative, IGF-I receptor positive human breast cancers.

Factors influencing tissue IGF-I bioactivity

It is logical to propose that IGF-I-receptor positive breast cancer cells are influenced by tissue IGF-I bioactivity in their microenvironment rather than by serum IGF-I concentration. Quantitative measurements of IGF-I concentration or bioactivity are not available for human breast cancer tissue, but it is widely believed that local IGF-I bioactivity is influenced by factors which include both systemic and local production rates of both IGF-I and IGF-binding proteins. IGF-I binding proteins are a diverse group of circulating and tissue proteins that have high affinity binding sites for somatomedins, and modulate binding interactions between these growth factors and their receptors [1].

Control of IGF-I levels

Factors controlling local production rates of IGF-I are incompletely described, but there is good evidence that the IGF-I gene is expressed by stromal cells of primary human breast cancers [19]. It is possible that stromal cells are capable of producing IGF-I not only in response to various physiological signals [20-24] (a stimulatory role for GH has not been excluded), but also in response to pathophysiological signals originating from neighbouring tumour cells [19]. There is no strong evidence that autocrine production of IGF-I is a common event in human breast cancer.

More is known about the factors regulating serum IGF-I level, which is strongly influenced by hepatic production of IGF-I. Growth hormone is the chief regulator of IGF-I gene transcription in the liver [25,26], but permissive roles for thyroid and/or steroid hormones have not been excluded [27,28]. In view of pituitary influences on each of these hormones, hypothalamic homeostatic mechanisms ultimately play a key role in regulating serum IGF-I levels.

Neuroendocrine mechanisms may be involved in the negative correlation between age and serum IGF-I level, which has been noted in several studies [29]. Multiple pathways may be involved in the reduction of IGF-I levels associated with malnutrition [30]. While one may speculate that the negative relationship between serum IGF-I level and caloric intake is related in some way to the body of data associating Western diet with breast cancer incidence [31], such a straightforward relationship is probably simplistic, as the differences of intake associated with variability in breast cancer incidence are considerably less than the relatively dramatic reductions of intake that have been associated with reduced IGF-I levels in experimental systems [32].

In view of the key role played by growth hormone in regulating IGF-I gene expression, it is important to examine factors that determine pituitary growth hormone output. Secretion of growth hormone by pituitary somatotrophs is regulated by a complex neuroendocrine control system involving the stimulatory (growth hormone releasing factor) and inhibitory (somatostatin) hypothalamic releasing factors. These interact to produce a dynamic pulsatile pattern of growth hormone release [33] . The influences of steroid hormones on the secretion of GH are complex [34]. The possibility that these relationships might be involved in the action of various

commonly used endocrine therapies for neoplasia has received little attention until recently. There is evidence that estrogens play at least a permissive role in pituitary release of GH [26,35-40]. In addition, there is evidence that sex steroids influence the expression of somatostatin and/or growth hormone releasing factor in the hypothalamus [41-43]. Estrogen receptors that might mediate this process are indeed present in the hypothalamic-pituitary axis [44]. The relevance of these experimental studies to clinical medicine is underlined by studies demonstrating that administration of estrogens to individuals with hypogonadism who have low levels of estrogen and subnormal levels of GH and IGF-I can substantially correct abnormalities in the GH/IGF-I axis seen in this condition [45], a result consistent with the view that estrogens play a permissive role for GH secretion.

Control of binding protein levels

Our knowledge concerning the regulation of serum and tissue levels of the various IGF binding proteins remains incomplete, and is the subject of active investigation in many laboratories [1,46,47]. It is clear that somatomedin activity is influenced not only by the complex neuroendocrine systems that regulate GH secretion, but also by local concentrations of binding proteins. The existence of multiple levels of physiological control of somatomedin activity is in keeping with the view that this activity plays a crucial role in regulating cellular proliferation. Discussion of the proposed physiological roles and regulation of expression of the various IGF binding proteins is beyond the scope of this review, but it should be emphasized for those not working in the field that the binding proteins have affinity for IGFs in the same range as membrane receptors and are not 'non-specific' carrier proteins. Roles of estrogens and/or antiestrogens in regulating their expression have not been well defined.

Effect of tamoxifen on serum IGF-I levels — clinical studies

In 1989, Colletti, Copeland, et al reported that mean serum IGF-I levels in a group of patients with metastatic breast cancer receiving tamoxifen was lower than that seen in a control group of breast cancer patients taking a variety of other antineoplastic treatments [48]. The patient population in this study was not uniform. Nevertheless, while the difference between groups was modest, it was statistically significant.

We took advantage of the availability of patients enrolled in a randomized, blinded, placebo-controlled clinical trial of tamoxifen in adjuvant therapy of breast cancer (NSABP B14 [49]) to independently test the hypothesis that antiestrogens might suppress the GH/IGF-I axis in humans by blockade of estrogen receptors in the hypothalamic-pituitary axis [50]. This study involved 69 patients, and yielded results similar to those reported by Colletti. Multivariate analysis confirmed that tamoxifen use was a significant predictor of serum IGF-I level, as was age. Although the reduction of mean IGF-I levels was modest (0.9 u/ml vs. 1.4 u/ml), the difference was statistically significant. Furthermore, the distribution of IGF-I levels was quite different between tamoxifen and placebo groups: in the former group 4.1% of individuals had IGF-I levels greater than 2 u/ml and 16.7% had levels less than 0.5 u/ml, as compared to 23.8% over 2 u/ml and 4.8% under 0.5 u/ml in the placebo-treated controls.

Recently, in collaboration with Craig Jordan and Andreas Fridel, we have extended this observation further by measuring serum IGF-I levels in a cohort of patients before and during tamoxifen therapy [51]. We observed a modest but statistically significant ($p<.05$) decline in serum IGF-I level in individual patients following the

institution of tamoxifen therapy, a result consistent with the differences between groups taking placebo and tamoxifen in our previous study. In a small number of patients for whom multiple serum samples were available, we observed that the decline in IGF-I level associated with tamoxifen usage could persist for up to 5 years. Kiang et al [52] and Lien et al [53] have recently presented data which independently confirm the results of our randomized, placebo-controlled clinical study.

In all these studies, patient-to-patient heterogeneity with respect to the degree of suppression of IGF-I was noted. The lowest IGF-I levels were seen in post-menopausal women, who tended to have lower IGF-I levels to begin with, and to exhibit larger declines than younger women. Further clinical research now under way will attempt to determine whether there is any correlation between degree of decline of IGF-I and clinical response to treatment. While it is well known that older women tend to respond better to tamoxifen than younger women, the relationship between decline in IGF-I and response remains speculative at this time. Similarly, no data is available to test the hypothesis that relapse while on tamoxifen is related to escape from suppression of serum IGF-I level. Most work related to tamoxifen resistance has focused on events within neoplastic cells. While this line of research is promising, it does not exclude the possibility of 'host' factors contributing to tamoxifen resistance. There are a number of mechanisms by which such escape might be mediated, including increased GRH secretion and/or decreased somatostatin secretion. Further work is needed to evaluate this hypothesis.

Effect of tamoxifen on the GH/IGF-I axis — laboratory research

Recent experimental work with primary cultures of pituitary cells [54] provides support for the hypothesis that tamoxifen can directly inhibit pituitary growth hormone output. Similar experimental systems had previously been used to document the stimulatory and inhibitory influences of growth hormone releasing hormone (GRH) and somatostatin on somatotroph function [55]. We observed that clinically relevant concentrations of tamoxifen (1-10 μM) significantly reduced the baseline production of GH, as assayed by radioimmunoassay of pituitary culture conditioned medium, and markedly attenuated the ability of the cultured cells to respond to GRH. The concentrations of tamoxifen employed did not affect the viability of the cultured pituitary cells. These results are consistent with the view that tamoxifen can inhibit GH synthesis and release at the level of the somatotroph. We speculate that this inhibition may involve pituitary estrogen receptors [54].

Chronically cannulated free-moving rats have been used for studies of growth hormone physiology, as *in vivo* GH secretion is pulsatile, and serum GH levels vary significantly from hour to hour. While this experimental system had previously been used to examine the effects of sex steroids on GH output [56], the effect of antagonists to sex steroids had not been studied. Tamoxifen, when administered in the manner originally described by Jordan as inhibiting DMBA-induced mammary cancer [57], had potent inhibitory effects on GH secretion, which persisted for at least 7 weeks following the administration of two doses of 5 mg subcutaneously [58]. This prolonged inhibition may well reflect the slow release and long half life of tamoxifen administered in this manner [59], rather than indicating a sustained inhibition following a transient exposure to the drug. Of considerable interest is the fact that the inhibitory effect of tamoxifen on the *in vivo* secretion of GH could be attenuated by an anti-somatostatin antiserum [58]. This implies that at least a part of the inhibitory effect of tamoxifen on GH output may be

mediated by increased release of endogenous somatostatin, a concept that is consistent with earlier reports showing that somatostatin gene expression in the rat hypothalamus can be regulated by sex steroids. Recent studies using tritiated estradiol have provided morphological evidence of estrogen receptors in both the arcuate-ventromedial and preoptic regions [60], the majority of which were localized to GRH secreting cells. Therefore, it is possible that over and above the direct effects of tamoxifen on pituitary cells implied by our *in vitro* studies, tamoxifen has a negative influence on GRH secretion and a positive influences on somatostatin secretion at the hypothalamic level. Furthermore, direct actions of tamoxifen on IGF-I gene expression by hepatocytes and/or other cell types have not been excluded.

Discussion

Systemic effects of tamoxifen

Although tamoxifen is a commonly used drug [61], our understanding of its mode or modes of action remains incomplete. Over the past decade, work from Lippman's group and others has provided important information concerning the sequelae of tamoxifen binding to estrogen receptors present on breast cancer cells. Examples of such events include stimulation of expression of the inhibitory factor TGFβ and inhibition of expression of the stimulatory factor TGFα [62,63]. Clinical and experimental research reviewed here provides evidence that tamoxifen has, in addition to its effects at the level of the breast cancer cell, an inhibitory effect on the GH/IGF-I axis. This property of tamoxifen is not, of course, the only systemic endocrine effect of the drug. There is evidence that tamoxifen suppresses estrogen-dependent prolactin secretion by the pituitary gland [64], and data to suggest that in premenopausal women, tamoxifen can raise estrogen levels, presumably by blocking central estrogen receptors and interfering with feedback inhibition of gonadotrophin secretion [65].

While the characterization of a previously unrecognized effect of a drug as commonly used as tamoxifen is of interest, a key unanswered question concerns the relationship, if any, between suppression of the GH/IGF-I axis by tamoxifen and its antineoplastic properties. The degree of suppression of IGF-I levels associated with tamoxifen usage in humans is modest; it remains to be determined whether changes of this magnitude affect breast cancer proliferation. Co-administration of tamoxifen with compounds such as somatostatin analogues or GRH antagonists might be expected to suppress the GH/IGF-I axis more completely than tamoxifen alone [50,66,67]. A trial comparing the efficacy of tamoxifen with that of the combination of tamoxifen and a somatostatin analogue is underway at the Mayo Clinic, North Central Oncology Group, and McGill University.

This approach can be viewed as a step towards 'selective medical hypophysectomy'. By this, I refer to the pharmacological ablation of those pituitary functions which are non-essential to adult women, but which may nevertheless stimulate breast cancer proliferation. Candidate pituitary functions for inhibition include the GH/IGF-I axis, the LH/FSH/ovarian axis, and prolactin secretion, while ACTH and TSH are not targets. Recently, there has been interest in this concept that stems from the realization that growth hormone is an agonist for the prolactin receptor in humans [68]; thus, the older clinical trials [69] showing no effect of pharmacologically reducing prolactin secretion alone in human breast cancer were doomed to fail, even if lactogenic hormones do play a stimulatory role. This has led to at least one trial, currently under way, which is exploring the simultaneous pharmacological inhibition of prolactin and growth hormone secretion [70].

Research regarding the effect of tamoxifen on the GH/IGF-I axis now provides independent justification for further research in this area. Although there is significant species-to-species and individual-to-individual variability in central actions of tamoxifen, the drug clearly can, at least in certain experimental systems, reduce both prolactin [64] and growth hormone secretion [58], over and above its action at the estrogen receptors of neoplastic cells. Detailed quantification of these effects in humans, and information concerning their relevance to the antineoplastic actions of the drug, are unavailable. If the central actions of tamoxifen do contribute to the antineoplastic actions of the drug in humans, it is possible that enhancing these effects by combining tamoxifen with agents such as LHRH antagonists and/or somatostatin analogues will be of therapeutic value. It is also possible that variables related to pituitary function represent risk factors and/or prognostic factors for breast cancer.

Some older literature deserves re-examination in this context. Reports documenting responses of metastatic breast cancer to surgical hypophysectomy even in the setting of prior oophorectomy and adrenalectomy [71] are consistent with a role for the GH/IGF-I axis. Evidence consistent with the view that the stimulatory effect of estrogens on breast cancer cell proliferation involves the pituitary gland is found in classic studies showing that estrogens are incapable of stimulating *in vivo* proliferation of estrogen receptor positive DMBA-induced tumours in hypophysectomized rats [72]. While there is reason to interpret this result as evidence that estrogen-stimulated prolactin release by the pituitary is an important way in which estrogen stimulates neoplastic proliferation in the DMBA model [73], it should be recalled that DMBA-induced tumours are IGF-I-receptor positive [74], and this result is also consistent with a role for the GH/IGF-I axis in the *in vivo* proliferation of DMBA-induced tumours.

Multiple levels of interaction between estrogens and IGF-I

There may be multiple levels at which estrogens (and antiestrogens) influence IGF-I physiology. In addition to the interactions emphasized in this review, there is laboratory evidence that expression of type I IGF-I receptors by ER positive breast cancer cells is up-regulated by exposure to estrogens [75], and down-regulated by antiestrogens [76]. Thus tamoxifen may not only reduce circulating IGF-I levels but also reduce responsivity of the neoplastic cells to this mitogen, in addition to blocking any proliferation that is directly dependent on estrogens. This laboratory result is consistent with the observation that there is a positive correlation between estrogen receptor level and IGF-I receptor level in primary human breast cancer [13]. Those tumours with an ER negative, IGF-I-R positive phenotype may arise from abnormal regulation of IGF-I-R expression.

Role of circulating IGF-I in adults

It is well known that patients who undergo surgical hypophysectomy for pituitary tumours require replacement of thyroid, adrenocortical, and often sex steroid hormones. In the adult, deficiency of GH and circulating IGF-I do not appear to be associated with major symptoms [77], although there is evidence that certain changes associated with aging may be related to the well described gradual decline of IGF-I levels with age [78]. In contrast, subnormal function of the GH-pituitary axis during growth and development is associated with well described pathology [79].

It would be fortuitous indeed if a subset of human malignancies exhibited a dependence on exogenous IGF-I, as novel endocrine therapies could exploit such a situation with minimal adverse effect on the patient. Perhaps tamoxifen as currently used acts in this way to a certain extent, not only in breast cancer, but also in certain desmoid [80] and pancreatic [81] tumours.

Neoplasms that are IGF-I-receptor negative and those that are IGF-I-receptor positive but express the IGF-I gene in an autocrine fashion would clearly not be candidates for this kind of treatment. A significant number of breast or other neoplasms [82], however, may respond to IGF-I from endocrine (circulating) or paracrine (e.g. stromal cell) sources. It is known that the former is growth hormone-dependent [83], and it will be important to extend previous work [20-24] to examine factors that regulate paracrine IGF-I expression, with particular attention to the role, if any, of growth hormone.

Effects of tamoxifen on serum lipids

A beneficial effect of tamoxifen on serum lipid levels has been described [84], and this effect may be of major clinical importance, particularly if ongoing trials of the drug lead to its widespread use as a preventative agent. The mechanism by which tamoxifen acts to alter lipid profiles remains incompletely characterized. As there is data to suggest that growth hormone raises serum triglyceride and cholesterol levels [85], it is possible that the growth hormone suppressive action of tamoxifen that we have reviewed here contributes to the lipid-lowering effects of the drug.

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