Evaluation of Continuous Infusion Suramin in Metastatic Breast Cancer: Impact on Plasma Levels of Insulin-like Growth Factors (IGFs) and IGF-binding Proteins

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ABSTRACT

Suramin represents a new class of antitumor drugs that targets growth factor networks. In this Phase II trial, suramin was administered by continuous infusion to 10 patients with advanced breast cancer. The target level of 280 μg/ml suramin was achieved in a median of 10 days; toxicities in this patient group were low. We monitored the insulin-like growth factor (IGF) network in these patients because of the previously defined growth-promoting role of the IGFs in breast cancer. Plasma levels of total IGF-I and total IGF-II showed variable responses to suramin with median decreases of 24 and 23%, respectively, for the 10 patients; for total IGF-I levels, this did not reach statistical significance. On the other hand, free IGF-I plasma levels were consistently and dramatically increased (over 250%) after suramin infusion. IGF-binding proteins (IGFBPs), modulators of IGF bioavailability, were also measured. Levels of IGFBP-3, the major carrier of IGFs in the circulation, were decreased 21% after suramin treatment when measured by immunoradiometric assay. However, the majority of the plasma IGFBP-3 remaining after suramin was not the intact high-affinity IGF-binding form but rather a 30-kDa fragment with markedly reduced affinity for IGF-I. IGFBP-3 protease activity was evident in the plasma of 3 of 10 patients after suramin. Measurements of plasma IGFBP-1, IGFBP-2, and IGFBP-4 revealed no significant changes in response to suramin. The dramatic increase in active free IGF-I seen after suramin raises concern and underscores the importance of measuring relevant biomarkers in clinical trials.

INTRODUCTION

Breast cancer is a leading cause of death, and its treatment produces significant morbidity for hundreds of thousands of women annually. Innovative therapeutic approaches for breast cancer are needed, especially for those cancers unresponsive to current hormonal and chemotherapeutic strategies. Breast carcinogenesis has been shown to be dependent on a complex autocrine and paracrine network of growth factors, and attempts to exert an antitumor effect through manipulation of peptide growth factors represent one new therapeutic approach.

Multiple reports over the last decade have established IGF-I and IGF-II as important mitogens for transformed cells and tissues, including breast cancer (1-3). The IGFs exert their mitogenic action through binding to IGF-I receptors on the plasma membrane of cells (4). This receptor is expressed in breast cancer cell lines and tumor biopsies (5). Activation of the IGF-I receptor by IGFs initiates a mitogenic signal transduction cascade. This response in breast cancer cell lines can be inhibited by antisense RNA or antibody specific to the IGF-I receptor (6, 7). Arteaga et al. (8) reported that breast cancer tumor growth in nude mice could be inhibited by a dose-dependent manner by IGF-I receptor antibody. This antibody also inhibited the growth of T6O breast cancer xenografts (9). Because the IGFs serve as potent activators of breast cancer cell growth in vivo and in vitro, the ability to regulate these mitogens may well provide a means to moderate breast cancer cell growth.

Suramin represents a new class of antitumor drugs that targets growth factor networks. Although suramin’s mechanism of action is not completely understood, in vitro studies have shown that it inhibits the binding of peptide growth factors, including IGFs, to their receptors (10, 11). Suramin has shown promising antineoplastic effects on several tumor types, including prostate and adrenocortical malignancies and some lymphomas (12-14). In breast cancer, suramin has growth-inhibitory effects in vitro in both hormone-insensitive ER-negative breast cancer cell lines and hormone-responsive ER-positive breast cancer cells (15). In these cultured cells, suramin inhibited IGF-I- and IGF-II-stimulated proliferation in a time- and dose-dependent manner.

In a pilot study involving eight patients with metastatic cancer resistant to standard therapy, including four patients with...
breast cancer, Miglietta et al. (16) observed a significant inverse relationship between the plasma levels of suramin and the levels of total IGF-I and IGF-II. These results are similar to the effects of treatment with the antiestrogen tamoxifen. Tamoxifen, an effective hormonal agent in ER-positive breast cancers, has been shown to decrease total IGF-I concentrations in women with advanced breast cancer (17, 18).

Total IGF concentrations represent IGFs in association with specific IGFBPs, seven of which have been identified to date (19, 20). Greater than 90% of plasma IGFs are tightly bound to IGFBP-3 and an ALS within a high molecular weight complex of ~150 kDa that prevents extravascular transit. IGFs not captured in the ternary complex can traverse the capillary endothelium and are available to local tissues (21). Thus, this portion of circulating IGFs may be biologically important.

Given the recognized importance of growth factor networks in breast cancer and suramin’s antitumor activity in vitro, we initiated a Phase II study of suramin in patients with minimally pretreated advanced breast cancer. In this study, we measured IGFs and IGFBPs in the plasma of patients both before and after suramin infusion to assess the potential impact of this treatment on IGF bioavailability. Our data underscore the importance of monitoring relevant biomarkers in clinical trials and confirm the complexity of the IGF system in human breast cancer.

MATERIALS AND METHODS

Eligibility Criteria. Eligible patients had histologically or cytologically confirmed advanced breast cancer; measurable disease; Eastern Cooperative Oncology Group performance scores of 0, 1, or 2; life expectancy of at least 3 months; WBC counts ≥3000 mm<sup>3</sup>; platelets ≥150,000 mm<sup>3</sup>; plasma creatinine ≤1.5 mg/dl; creatinine clearance ≥60 ml/min; aspartate aminotransferase/alanine aminotransferase <2× normal; and a direct plasma bilirubin ≤0.3 mg/dl. Patients were excluded from participation if they had any of the following: rapidly progressive or life-threatening disease; more than one prior chemotherapy regimen for metastatic disease; prior chemotherapy within the preceding 4 weeks (6 weeks for mitomycin C or nitrodiy); prior radiation within the preceding 2 weeks; brain metastases; previous life-threatening malignancy; plasma calcium >12 mg/dl; or a serious intercurrent medical illness, including significant cardiac disease, a bleeding diathesis, a prior history of cerebrovascular accident, concurrent use of coumadin or heparin, significant peripheral neuropathy, or central nervous system disease. Pregnant or nursing females were excluded from this study. All patients signed an informed consent form. Concomitant radiation therapy, chemotherapy, immunotherapy, anticoagulants, or calcium channel blockers were not allowed.

Suramin Dosing and Treatment Evaluation. The clinical objectives of the study were to assess the antitumor efficacy and toxicity of suramin in patients with metastatic breast cancer. The treatment plan was as follows: all patients received an initial loading cycle of suramin; for all patients with responding or stable disease, a second loading cycle was to be administered 2 months after the initiation of cycle 1; and after those initial two cycles of therapy, all patients were restaged. Subsequently, only patients experiencing clinical benefit were to be continued on suramin therapy.

The objective response criteria were as follows: complete regression, disappearance of all evidence of tumor; partial regression, 50% or greater reduction in the product of the longest perpendicular diameters of the indicator lesion(s); stable, failure to qualify for complete regression, partial regression, or progression; and progression, increase of 25% in the product of the longest perpendicular diameters of the indicator lesion(s) or the appearance of new lesions.

Suramin (NCS 3496) was provided by the Pharmaceutical Resources Branch, National Cancer Institute (Bethesda, MD). The first day’s dose was divided into an initial test dose of 200 mg given i.v. over 10 min (in 50 ml of dextrose 5% in water), which was followed after 20 min with the initiation of a continuous infusion at 350 mg/m<sup>2</sup>/day. Plasma suramin concentrations were measured starting on day 3, and the subsequent rate of suramin infusion was adjusted to reach a desired suramin level of 280–300 μg/ml. One cycle was completed at this point of suramin infusion. Because of suramin’s tendency to cause adrenal cortical damage, all patients were started on oral hydrocortisone, 25 mg every a.m. and 15 mg every p.m. at the time of initiation of suramin treatment.

Plasma. Plasma samples were obtained daily from each patient during suramin infusion. EDTA was used as the anticoagulant. IGF studies were performed on sera presuramin and after plasma suramin levels reached 280 μg/ml. Plasma was stored at −70°C until use.

High-Performance Liquid Chromatography Assay. Suramin plasma concentrations were determined by the reverse-phase high-performance liquid chromatography procedure of Supko and Malspeis (22). Separations were achieved on a Nova-Pak C<sub>18</sub> Radial-Pak cartridge column (100 × 5 mm; inside diameter, 4 μm) installed in an RCM cartridge holder. A guard-Pack Nova-Pak C<sub>18</sub> precolumn insert was placed before the analytical column. The mobile phase consisted of 49% methanol and 51% of an aqueous solution of 19.6 mM ammonium acetate and 1.96 mM tetrabutylammonium phosphate (pH 6.5). UV absorbance of suramin was monitored at 238 nm. To 25 μl of patient plasma was added 500 μl of acetonitrile containing 50 mM tetrabutylammonium phosphate and 5 μg of the internal standard 2-naphthol. After rapid mixing for 30 s and centrifugation (10 min at 15,000 × g), 200 μl of the supernatant were combined with 200 μl of 10 mM ammonium acetate, and 10 μl were injected onto the column.

IRMAs. Levels of IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 were measured in plasma using specific and highly sensitive two-site IRMAs (Diagnostic Systems Laboratories, Webster, TX). For determination of total IGF concentrations, samples were acid/ethanol-extracted to separate IGF/IGFBP complexes and remove IGFBPs before assay. Unextracted plasma were used for determination of free IGF-I. Free IGF is defined as IGF not bound by or readily dissociable from IGFBPs. Because suramin inhibits the binding of IGFs to IGF receptors (10, 11), we needed to determine whether suramin would interfere with the binding of IGFs to antibodies in the assays. When tested with samples in all six IRMAs, direct addition of suramin at the maximal levels achieved in plasma (300 μg/ml) did not interfere in the assays. The free IGF-I...
Table 1 Patient characteristics

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Lag years between primary diagnosis and initiation of suramin.
PS, Eastern Cooperative Oncology Group performance score.
ER, last available ER status before initiation of suramin (fmol/mg cytosolic protein, ligand-binding assay).
CMF, cyclophosphamide, methotrexate, 5-fluorouracil.
TAM, tamoxifen.
ADR, Adriamycin.
IFL-RA, interferon a.
DES, diethylstilbestrol.
HAL, halotestin.
CAF, cyclophosphamide, Adriamycin, 5-fluorouracil.
6-TG, 6-thioguanine.

IRMA had a lower detection limit of 0.03 ng/ml and a CV of 10% at 0.3 ng/ml. The total IGF-I IRMA had a lower detection limit of 0.08 ng/ml and a CV of 3.0% at 55 ng/ml. The total IGF-II IRMA had a lower detection limit of 0.13 ng/ml and a CV of 4.3% at 416 ng/ml. The IGFBP-1 IRMA had a lower detection limit of 0.11 ng/ml and a CV of 4.0% at 10 ng/ml. No lower detection limit or CV was available for the IGFBP-2 IRMA. The IGFBP-3 IRMA had a lower detection limit of 0.5 ng/ml and a CV of 2% at 83 ng/ml.

Western Ligand Blot. Western ligand blotting was performed as described previously (23). Plasma (1 μl) was subjected to SDS-PAGE under nonreducing conditions. The proteins were transferred to nitrocellulose using a semidyed transfer apparatus. The nitrocellulose filters were blocked with 0.5% BSA and incubated with [125I] labeled IGF-I overnight at 4°C. The blots were washed, dried, and exposed to either phosphor plates for PhosphorImager analysis and quantitation (Molecular Dynamics) or to film for autoradiography.

Western Immunoblot. Plasma (0.5 μl) was electrophoresed, transferred to nitrocellulose, and blocked as described above. The filters were incubated overnight at 4°C with IGFBP-3 antibody [a generous gift of Dr. R. G. Rosenfeld (Oregon Health Science University, Portland, OR)] at 1:1000 final dilution. The filters were washed and incubated for 1 h with horseradish peroxidase-linked goat antirabbit antibody at room temperature. The filters were again washed and developed in enhanced chemiluminescence reagent (Amersham, Chicago, IL) and exposed to film.

Protease Assays. IGFBP-3 protease activity was determined by the method of Lamson et al. (24). Briefly, 1 μl of plasma was incubated overnight at 37°C with [125I]-labeled IGFBP-3 in 25 μl of 1 mM CaCl2 and 50 mM Tris buffer (pH 7.4). The samples were subjected to SDS-PAGE under reducing conditions. The gels were dried and exposed to either phosphor plates or film as in the Western ligand blot analysis.

Statistical Methods. For each biomarker, the Wilcoxon signed rank test was used to assess whether the percentage change in peak levels from pretreatment levels was significantly different from zero.

RESULTS
Clinical End Points. Ten patients were enrolled in the trial, and their characteristics are outlined in Table 1. The target level of suramin (median, 284 μg/ml; range, 239–319 μg/ml) was achieved after a median of 10 days (range, 7–19 days) of continuous suramin infusion. By careful monitoring during the loading course, there was only modest (CV < 10%) variability in the concentration. One patient had a partial response (at least a 50% reduction in the product of the longest perpendicular diameter) in a peripheral lymph node lasting 85 days. Another patient experienced clinical stabilization of painful, progressive bone disease for 9 months. Another was found to have brain metastases (persistent headaches) on day 9 of her first cycle of suramin; this patient was removed from the study at that time with a suramin level of 239 μg/ml. She was not evaluable for clinical end points but was included in the pre- and postsuramin biomarker analyses. The clinical outcomes of treatment effect, number of cycles of suramin administered, time to progression, and time to death are provided in Table 2. Overall, the toxicities seen in this patient group with carefully monitored blood levels were tolerable (Table 3). The most common side effect was a pruritic maculopapular rash. No lasting severe side effects were encountered.

Pharmacokinetics. The pharmacokinetics of suramin were characterized for three patients from whom plasma specimens were obtained during and after the continuous infusion. Modeling of suramin disposition to a two-compartment open model indicated slow plasma clearance (0.0112 liter/h/m²) and...
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The median pretreatment level was 129 ng/ml. Six patients had a lower total IGF-I level after suramin treatment, and four patients, total IGF-II levels decreased after suramin treatment. The tenth patient’s IGF-II level remained essentially unchanged.

The percentage decrease in total IGF-II levels from pretreatment values ranged from 70 to 1384%. This increase was highly significant (P = 0.002). The free IGF-I values likely reflect true unbound IGF-I as assessed by specific IRMA. All biomarker analyses were performed on first-cycle specimens.

Assays for total IGF measure unbound IGFs and IGFs bound to IGFBPs. The median level of total IGF-II in the 10 patients before drug treatment was 832 ng/ml. In 9 of 10 patients, total IGF-II levels decreased after suramin treatment. The tenth patient’s IGF-II level remained essentially unchanged. The percentage decrease in total IGF-II levels from pretreatment values was significant (P = 0.004), ranging from −66 to 4%. Total IGF-I levels were highly variable among the 10 patients. The median pretreatment level was 129 ng/ml. Six patients had a lower total IGF-I level after suramin treatment, and four patients had an increased total IGF-I level. The percentage change in total IGF-I levels from pretreatment values was not found to be significant (P = 0.131).

The free IGF-I values likely reflect true unbound IGF-I as well as IGF-I that readily dissociates from IGFBPs. The free IGF-I levels of all 10 patients increased markedly after suramin treatment. The median baseline free IGF-I concentration was 0.2 ng/ml. The percentage increase in free IGF-I levels from pretreatment values ranged from 70 to 1384%. This increase was highly significant (P = 0.002).

IGFBP levels were also measured by specific IRMAs (Table 4). The IGFBP-1 and IGFBP-2 levels showed considerable intragroup variation and no significant change in response to suramin. Absolute plasma IGFBP-3 concentrations were within the range for healthy adults and decreased after suramin treatment in nine of the patients. The median decrease for the patient group was 21% (P = 0.010).

Assessment of immunoreactive IGFBP by IRMA alone gives insufficient information on the number and forms of IGFBP in the circulation. To further analyze the binding protein levels, the plasma samples were analyzed by Western ligand blotting (Fig. 1). Western ligand blotting separates proteins by apparent molecular size and measures the ability of the proteins to bind [125I]-labeled IGF-I after transfer to nitrocellulose filters. The bands at 42- and 38-kDa represent the expected glycosylation variants of IGFBP-3 (22, 23). The band at 34 kDa represents IGFBP-2, and the lowest band on the blot indicates 24-kDa IGFBP-4. The intensities of these bands were quantified using PhosphorImager analysis. The level of IGFBP-2 in each sample varied but related to the value obtained by IRMA, and there was no consistent change with suramin treatment. By Western ligand blot analysis, IGFBP-4 concentrations decreased modestly in 8 of 10 patients, and the overall change for the group was not remarkable.

Of particular interest was the finding that levels of IGFBP-3 detected by Western ligand blotting were markedly reduced in the plasma after suramin treatment (Fig. 1). In some patients, there was almost no detectable 38-42-kDa IGFBP-3 postsuramin treatment. These results are in contrast to the IRMA IGFBP-3 values for these patients, which suggested a moderate decrease in IGFBP-3 levels posttreatment. The addition of suramin at a final concentration of 300 μg/ml to control plasma samples had no effect on IGFBP assessment by Western ligand blotting (data not shown).

To address the apparent discrepancy between the IRMA and Western ligand blot results, we assayed the plasma samples by Western immunoblot using a specific IGFBP-3 antibody. This technique can detect IGF-binding and nonbinding forms of IGFBP-3. In all 10 of the study patients, the level of intact 38-42-kDa IGFBP-3 was greatly reduced after suramin treatment (Fig. 2). Some patients’ plasma had no detectable intact IGFBP-3 after the drug treatment. A 30-kDa immunoreactive IGFBP-3 form was evident in all of the plasma samples, however, both pre- and posttreatment. This form of IGFBP-3 was not detected by Western ligand blotting with [125I]-labeled IGF-I. In 5 of 10 patients, 30-kDa IGFBP-3 increased after suramin treatment. This fragment is similar to a 30-kDa IGFBP-3 fragment generated by a IGFBP-3 protease found in the plasma of pregnant women (Fig. 2, Lanes 3 and 4).

To elucidate a possible mechanism for the decreased level of intact IGFBP-3, IGFBP-3 protease activity was measured in the plasma by adding exogenous [125I]-labeled IGFBP-3 and monitoring the appearance of radiolabeled fragments. None of the patients’ pretreatment plasma samples produced IGFBP-3...
factor receptors and/or the relevant ligands has been shown in patients with advanced breast cancer, pre- and postexposure to suramin. We examined several components of the IGF system in our patients to define the predominant activity within the IGF network. Hence, we were concerned that the measure-

tumors, especially breast cancer (1-3). Recognizing the complexity of the IGF family, we were concerned that the measure-

ment of total IGF levels alone was insufficient to characterize the growth-promoting role of the IGFs in human solid tumors, especially breast cancer (1-3). Overexpression of specific growth factor receptors and/or the relevant ligands has been shown in vitro, in vivo, and in clinical models to enhance tumor growth or correlate with worse outcome (1, 2). In an attempt to target these growth factor pathways in breast cancer, we conducted a clinical trial of suramin, a prototype agent within the class of growth factor inhibitors that is thought to act generally to inhibit the binding of growth factors to their receptors (10). We monitored the IGF network in these patients because of the previously defined growth-promoting role of the IGFs in human solid tumors, especially breast cancer (1-3). Recognizing the complexity of the IGF family, we were concerned that the measurement of total IGF levels alone was insufficient to characterize the predominant activity within the IGF network. Hence, we examined several components of the IGF system in our patients with advanced breast cancer, pre- and postexposure to suramin.

The most striking result obtained in this study indicated that whereas total levels of plasma IGF-I decreased, but not significantly, and IGF-II decreased 23%, free IGF-I increased by 70–1384% after suramin treatment. An increase in free IGF-I was seen in all 10 patients. The decrease in the total IGF-II plasma levels agrees with a previous suramin trial (16) and was not due to direct effects of suramin in the assays. It is interesting to note that levels of the IGFs and IGFBPs measured in patients B and E, who had a stabilized condition and a partial response after suramin treatment, did not show any significant change from the median values. Also, the magnitude of the suramin-induced decrease in total IGFs is similar to the previously reported effect of tamoxifen on IGF levels. This antiestrogen reduced total systemic IGF-I concentrations in breast cancer patients by about 35% in two clinical trials (17, 18). However, unlike suramin, tamoxifen treatment resulted in a parallel decrease in levels of free IGF-I.4 Although the absolute levels of free IGF-I represent only 1% of total circulating IGF-I, it is the free form that is readily available to extravascular tissues and, at these concentrations, bioactive at the cellular level (26). IGFs in the picomolar to nanomolar concentration range have been reported to be potent mitogens in a variety of breast cancer cells (27).

In an effort to elucidate the mechanism responsible for the

proteolysis, whereas the postsuramin treatment samples of patients A, B, and F did exhibit low levels of an IGFBP-3 protease activity similar to that seen in plasma from women in late pregnancy. Suramin alone did not induce exogenous or endog-

enous IGFBP-3 proteolysis (data not shown).

DISCUSSION

Over the past decade, numerous lines of evidence have shown the central importance of peptide growth factor networks in human breast cancer. Overexpression of specific growth factor receptors and/or the relevant ligands has been shown in vitro, in vivo, and in clinical models to enhance tumor growth or correlate with worse outcome (1, 2). In an attempt to target these growth factor pathways in breast cancer, we conducted a clinical trial of suramin, a prototype agent within the class of growth factor inhibitors that is thought to act generally to inhibit the binding of growth factors to their receptors (10). We monitored the IGF network in these patients because of the previously defined growth-promoting role of the IGFs in human solid tumors, especially breast cancer (1-3). Recognizing the complexity of the IGF family, we were concerned that the measurement of total IGF levels alone was insufficient to characterize the predominant activity within the IGF network. Hence, we examined several components of the IGF system in our patients with advanced breast cancer, pre- and postexposure to suramin.

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In an effort to elucidate the mechanism responsible for the

dramatic rise in free IGF-I after suramin exposure, we determined the plasma levels of the circulating IGFBPs. After suramin treatment, plasma levels of IGFBP-3, the major carrier of the IGFs in plasma, decreased a modest 21% when measured by IRMA. However, when assessed by Western ligand blot, the levels of intact 38-42-kDa IGFBP-3 were dramatically decreased, in some instances, to almost undetectable levels. Immunoblotting with a specific IGFBP-3 antibody indicated the presence of a 30-kDa IGFBP-3 fragment, which did not bind $^{125}$I-labeled IGF-I by Western ligand blotting. Both the IGFBP-3 fragment with reduced affinity for IGF-I as well as intact IGFBP-3 with high affinity for IGF-I are measured in the IGFBP-3 IRMA. Suramin treatment produced no significant changes in IGFBP-1, IGFBP-2, or IGFBP-4 when assessed by IRMA or by Western ligand blotting. These IGFBPs are acutely regulated by nutritional factors not controlled in this study (28), and the resultant variability might obscure any effect. Alterations in the levels and saturation of these IGFBPs could affect IGF delivery and bioactivity (see below) and will need to be addressed in future studies.

What are the possible implications of a decrease in intact IGFBP-3 and an increase in free IGF-I in patients with breast cancer? One concern is that this could reflect an alteration in IGF availability to target tissues, including tumor. The majority of the circulating IGFs are bound with high affinity to IGFBP-3 in a 150-kDa ternary complex that includes an ALS. IGFs tightly bound in this ternary complex are generally latent and cannot cross the capillary boundary (21). Destabilization of the ternary complex results in redistribution of IGFs into the unbound form as well as into binary IGFBP complexes, which are cleared more rapidly from the circulation (29, 30). This increased transcapillary transfer of free, active IGF to target tissues could conceivably promote tumor cell growth. Indeed, alterations in IGFBP-3 may be more important for IGF delivery to tumor cells than alterations in total plasma IGF concentrations. In fact, it has been proposed that free IGF is increased to the human fetus by such a mechanism. IGFBP-3 modified by a specific protease induced in the maternal circulation during pregnancy has decreased affinity for IGF-I, thus potentially increasing bioavailability of IGF-I to the fetus (30, 31). Suramin’s long half-life could conceivably alter growth factor equilibria for protracted periods. Thus, systemic effects of suramin to decrease the high-affinity form of IGFBP-3 in the circulation might have an adverse effect in breast cancer patients, allowing increased free IGF availability and potential stimulation of tumor cells. Suramin has been reported to have stimulatory effects on tumor growth in rabbits (32), although effects on the IGF system were not examined in that study. We saw no evidence of a tumor-stimulating effect in our study, but the sample size was small.

Our data do not provide any information on the effect of suramin on the IGF system at the tissue level. However, in vitro studies have shown that, at high concentrations, suramin inhibits IGF/receptor interactions but that suramin’s effects are reversible. Wilms’ tumor growth is inhibited by suramin via interfering with IGF-II binding, but this inhibitory effect of suramin is overcome with excess IGF-II (11). Moreover, Pommier et al. (33) showed that at low concentrations that do not inhibit receptor binding, suramin induces release of IGF-II from local IGFBPs and actually facilitates IGF-I binding to receptors on colon cancer cells.

The mechanism by which suramin administration disrupts the ternary IGFBP-3/IGF/ALS complex is unclear. Suramin could inhibit IGF and IGFBP interaction in vivo as has been shown in vitro, leading to a general increase in free IGF. However, its particular effect to decrease intact IGFBP-3 suggests a more specific mode of action. IGFBP-3 protease activity has been widely studied and is especially active in the plasma of pregnant women. This protease clips intact IGFBP-3 into a 30-kDa fragment that has a much lower affinity for IGFs, resulting in increased IGF bioavailability (26, 31). Suramin did not directly act as an IGFBP-3 protease when tested in our cell-free assay (data not shown). Suramin treatment was associated with circulating IGFBP-3 protease activity in 3 of 10 patient plasma samples. This activity produced an IGFBP-3 fragment similar in size to that seen in pregnancy plasma. In addition, there may be peripheral proteolysis of IGFBP-3 with subsequent appearance of the fragment in the circulation. Lalou et al. (34) have reported on a cell surface-associated plasminogen/plasminogen activator system involved in IGFBP-3 proteolysis. This type of a cell-associated IGFBP-3 proteolysis would not be measured by our assay but would be consistent with the increase in immunoreactive 30-kDa IGFBP-3 in the plasma.

Although not directly measured in this study, low ALS
expression could also lead to a loss of intact IGFBP-3 and an increase in free IGF. ALS, when part of the ternary complex, stabilizes both IGFBP-3 and the bound IGF. Bang et al. (31) found that despite the prominence of the 30-kDa IGFBP-3 fragment, IGFBP-3 protease activity was not increased in fetal serum. Instead, they found a lower level of ALS in fetal serum in conjunction with an increased level of the 30-kDa IGFBP-3 fragment. More studies will be necessary to determine the underlying mechanism for the altered IGF system with suramin treatment.

In addition, we must consider a possible effect of hydrocortisone on the IGF system. Because of suramin’s known adrenal toxicity, all patients receiving suramin were also treated with oral hydrocortisone. Glucocorticoid could independently affect the levels of IGFs and IGFBPs. However, previous reports have shown that serum levels of IGFBP-3 increase after glucocorticoid treatment (35). Moreover, glucocorticoids have been shown either to have no effect on total IGF-I levels or to slightly elevate total IGF-I levels (36, 37).

Exploitation of the dependence of breast cancer cells on IGFs is a new and potentially important therapeutic approach. However, it is essential to consider the complexity and dynamics of the IGF system in designing such therapeutic strategies. Our experience with suramin in a group of women with minimally pretreated advanced breast cancer illustrates several challenges and provides motivation for further research regarding the biological significance of plasma IGF/IGFBP measurements. We would recommend thorough study of relevant growth factor intermediates as suramin’s role in the clinic continues to be defined.

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REFERENCES


Evaluation of continuous infusion suramin in metastatic breast cancer: impact on plasma levels of insulin-like growth factors (IGFs) and IGF-binding proteins.

J B Lawrence, C A Conover, T C Haddad, et al.


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