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SURAMIN, A NOVEL ANTITUMOR COMPOUND

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Summary—Suramin, a polyanionic compound originally synthesized for use as an antiparasitic agent, has recently entered clinical trials for the treatment of a variety of human cancers refractory to conventional modalities of therapy. This is based on suramin's ability to bind and to inactivate growth factor and enzyme systems critical to cellular homeostasis and proliferation. In addition, this compound possesses adrenocorticolytic properties *in vivo* and exerts significant cytostatic and cytocidal effects against a variety of human tumor cell lines *in vitro*. Pilot studies using suramin have thus far been conducted in adrenocortical carcinoma, prostate cancer refractory to conventional hormonal manipulation and nodular lymphomas.

INTRODUCTION

A major focus of cancer research in the course of the last 25 years has centered around the genetic alterations within the cellular genome that lead to unregulated cellular proliferation. The development of antineoplastic agents in this period has attempted to exploit the fact that some malignant cells proliferate more rapidly than surrounding normal tissues, and has primarily focused on compounds which interfere with DNA replication. In fact, to date, among the cytotoxic agents which have proven most efficacious in the treatment of cancer are those which interfere with the synthesis of normal DNA precursors, chemically interact with DNA itself or disrupt the mitotic spindle apparatus. More recently, the importance of peptide growth factors in many critical developmental phenomena in both malignant and normal tissues has been brought into light. Twelve years ago, De Larco and Todaro [1] first suggested that the endogenous production of growth factors, which act on their producer cells via functional receptors, could be fundamental in the process of malignant transformation. To date, numerous proto-oncogenes, including c-*sis* and *hst*, have been shown to encode for proteins with a marked sequence homology to known growth factors [2, 3]. On the premise that interruption of the growth factor–receptor interaction both at a cell membrane, and possibly at an intracellular level, could have a profound effect on tumor cell proliferation, our group has been evaluating the potential antitumor activity of suramin, a synthetic polyanionic compound with avid protein growth factor- and enzyme-binding properties [4, 5].

HISTORICAL PERSPECTIVE—PHARMACOLOGY

Suramin is formally the hexasodium salt of carbonyl bis[8-(3-aminobenzamido)-4-methylbenzamido]-naphthalene-4,6,8-trisulfonate (M_r = 1429). It was originally synthesized by Bayer AG in 1916 following over a decade of research on the trypanocidal activity of the azo dyes trypan red and trypan blue [6, 7]. It has been used clinically both in the treatment of African trypanosomiasis and subsequently onchocerciasis [8]. The dose of suramin traditionally employed in the treatment of parasitic diseases is a 200 mg test dose, followed by weekly 1 g boluses for up to 6 weeks.

In the mid-1980s, this compound was briefly tested as a possible antiviral agent in the treatment of acquired immunodeficiency syndrome (AIDS) after it was shown that aside from inhibiting the activity of viral reverse transcriptase, the presence of suramin also blocked both the cytopathic effects and the infectivity of the human immunodeficiency virus (HIV) *in vitro* [9–11]. No clinical or immunologic improvement, however, could be documented in these patients.

Many of suramin's biologic and pharmcologic properties relate both to the spatial positioning of its six sulfonic acid groups and to the

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presence of aromatic rings. Protein binding results both as a consequence of its overall anionic charge and perhaps through ring stacking (i.e. binding to aromatic amino acids with proteins). Suramin's avid binding to plasma proteins, its significant tissue uptake (primarily by the renal parenchyma) and absence of known metabolism *in vivo*, result in an extremely slow total body clearance, with a terminal half-life in the range of 45–55 days [12, 13]. Urinary clearance of suramin appears to be the sole mode of drug elimination.

ADRENOCORTICOLYTIC PROPERTIES

An association between suramin therapy and the development of adrenal insufficiency was first postulated by researchers in the mid-1930s [14]. In 1987, our group administered 5 weekly bolus doses of suramin (800 mg/m^2) to 5 cynomolgus monkeys [15]. Progressive elevation of plasma ACTH levels compared to controls and decline in the cortisol response to ACTH stimulation were documented in the treated animals. Histopathologic evaluation of the adrenal glands from the treated animals revealed frank disruption of the architecture of the adrenal cortex, often with the presence of a diffuse inflammatory infiltrate. No abnormalities in the adrenal medulla were found.

We have extended these observations with our *in vitro* work with an adrenocortical carcinoma cell line, NCI-H295 [16]. This cell line, derived from an invasive primary carcinoma of the right adrenal gland, produces detectable levels of androgens, mineralocorticoids as well as glucocorticosteroids [17]. Exposure of this cell line to suramin 300 μg/ml resulted in a marked decline in steroid production (Fig. 1). These results are consistent with those reported by Ashby *et al.* [18], who demonstrated that suramin, at concentrations as low as 75 μg/ml, is able to inhibit by 50% the activity of steroidogenic enzymes in isolated adrenal mitochondrial and microsomal preparations. Included among these enzymes are 17β-hydroxylase, 17–20-desmolase and 21-hydroxylase. In view of suramin's adrenocorticolytic properties, all patients treated at the NCI are placed on replacement doses of hydrocortisone (20–30 mg/day) at the start of therapy.

GROWTH FACTOR AND ENZYME BINDING BY SURAMIN

Platelet-derived growth factor (PDGF) was the first mitogen shown to bind to suramin *in vitro* and to dissociate from its cell surface receptor with the addition of clinically achievable concentrations of the drug [19, 20]. Hosang [20] presented, in addition, evidence that suramin does not appear to interact to an appreciable extent with the PDGF receptor.

In 1987, Coffey *et al.* [21], in their work with the mouse embryonic clonal cell line AKR-2B, demonstrated that suramin was capable of

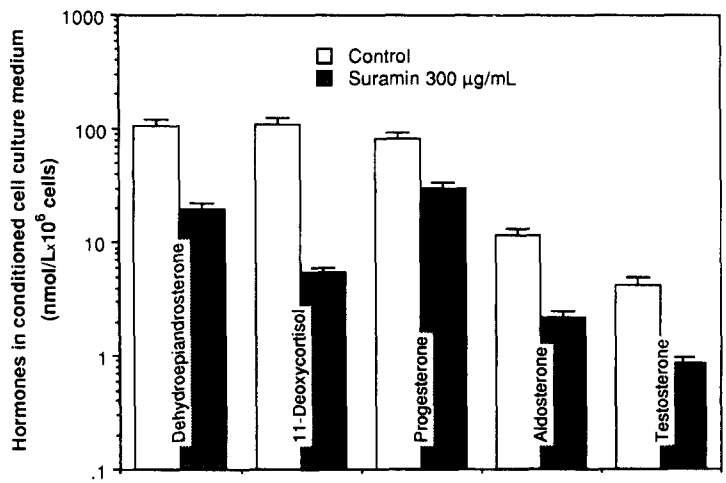

Fig. 1. Inhibition of steroid hormone production by NCI-H295 adrenocortical cancer cells treated with suramin. These cells (4×10^4/ml) were plated in medium containing 10% fetal calf serum, to which suramin 300 μg/ml was then added. After 144 h the cells were pelleted by centrifugation and the culture medium was collected and assayed for hormones as previously described [16]. The concentrations shown are obtained by subtracing steroid hormone levels in culture medium conditioned by NCI-H295 from those present in non-conditioned cell culture medium (which accounted for <4% of the control values). *Columns*: mean values of 2 experiments each in duplicate. Bars ± SEM.

disrupting the attachment of other heparin-binding growth factors to their respective cell surface receptors. Included among these are transforming growth factor β (TGF-β), basic fibroblast growth factor (bFGF) and, to a lesser degree, epidermal growth factor (EGF). These authors documented a direct correlation between inhibition of growth factor-receptor binding by suramin and decreased mitogenicity. In addition, they described an effective decrease in spontaneous colony formation in soft agar by AKR-MCA cells, a chemically transformed variant of AKR-2B cells in the presence of suramin 0.1 mM. Recently, suramin has been shown to reversibly interfere with the binding of insulin-like growth factor-I (IGF-I) to osteogenic sarcoma cells and to block IGF-I-stimulated proliferation of these cells [22].

The ability of suramin to prevent mitogen-receptor interaction may be the result of its binding directly to amino acids within the growth factor's receptor binding domain, or through attachment to other domains and thereby inducing a conformational change in the growth factor molecule that renders it unable to bind to its receptor.

Suramin's binding to a variety of cellular enzyme systems critical to cell proliferation and homeostasis, with consequent disruption of their function, may be the fundamental mechanism by which this compound exerts its antiproliferative effect. Included among the enzymes known to be inactivated by suramin *in vitro* are the DNA polymerases, terminal deoxynucleotidyl transferase (TdT), protein kinase C, various lysosomal and microsomal enzymes and the Na^+/K^+-, Ca^{2+}- and H^+-ATPases [8, 18, 23–26].

EFFECT OF SURAMIN ON HUMAN CANCER CELLS *IN VITRO*

Our group, as well as others, have evaluated the *in vitro* effect of suramin on a variety of human cancer cell lines [5, 23, 27]. The methodology and particular assay used in each study to measure growth inhibition and effective "cytotoxicity" are critical in comparing the results obtained. Two human adrenal cancer cell lines (SW-13 and NCI-H295) and three prostate cell lines (PC-3, DU 145 and LNCaP-FBC) are among those tested for sensitivity to suramin by our group [16, 28]. Colony formation in 10% fetal calf serum following a 144-h exposure to suramin was assayed by the subsequent harvesting and replating of a determined aliquot of the exposed cells in drug-free medium, with counting of the colonies formed after 12 days and comparison with control cells not exposed to surmain. The SW-13, PC-3 and LNCaP-FGC cell lines exhibited significant inhibition of cell proliferation and colony formation at concentrations of suramin that are clinically achievable in humans without excessive toxicity (150–300 μg/ml). The DU 145 and NCI-H295 cell lines remained relatively resistant to concentrations of suramin up to 400 μg/ml for 144 h. Increased inhibition of colony formation with still longer durations of suramin exposure is predicted. Additional tumor cell line types with *in vitro* sensitivity to suramin include sarcoma, glioblastoma, lymphoma, ovarian and melanoma.

The ability of shorter durations of suramin exposure to selectivity inhibit growth factor- and testosterone-induced mitogenicity of LNCaP-FGC cells was also documented [28]. Suramin, at a concentration of 300 μg/ml was able to inhibit bFGF-, but not EGF-induced stimulation of DNA synthesis in defined medium, and the mitogenic effect of testosterone measured by [*methyl*-^{3}H]thymidine incorporation (Fig. 2).

CLINICAL TRIALS

The major focus of our clinical study of suramin has been centered around the treatment of three malignancies: adrenocortical cancer; metastatic prostate cancer refractory to conventional hormonal manipulation; and, more recently, advanced nodular lymphomas requiring systemic therapy [16, 29, 30]. The rationale for the use of suramin in these malignancies is based upon the following observations:

(1) suramin is capable of blocking the activity of several growth factors, including bFGF, PDGF and TGF-β, which have been postulated to have an important role in tumor cell biology;
(2) suramin exerts a marked direct inhibitory effect on cell growth and colony formation of some cell lines of these tumor types at concentrations clinically achievable in humans without excessive toxicity;
(3) suramin destroys adrenal cortical cells *in vivo*; and
(4) suramin's adrenocorticolytic properties may slow prostate cancer cell proliferation

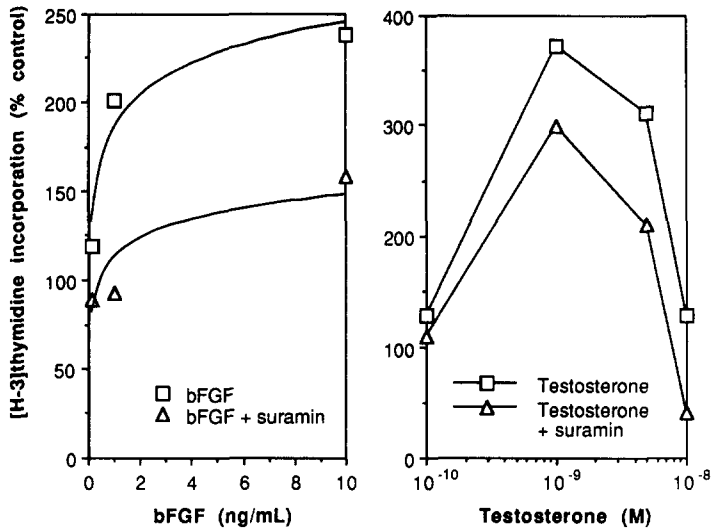

Fig. 2. Inhibition of bFGF- and testosterone-induced stimulation of DNA synthesis as measured by [*methyl*-^{3}H]thymidine incorporation by LNCaP-FGC prostate cancer cells. *Left panel.* Cells were plated in 24-well plates in medium containing 0.5% fetal calf serum. When they were confluent the cells were exposed to bFGF and 2 h thereafter suramin 300 μg/ml was added. 24 h later cells were pulsed with 1 μCi/ml of [*methyl*-^{3}H]thymidine for 2 h and the incorporated radioactivity was precipitated with 10% trichloroacetic acid. Cells were lysed with 0.25 M NaOH and an aliquot was counted in a liquid scintillartion counter and the incorporated radioactivity expressed as percentage of control. *Right panel.* Cells (1×10^4/cm^2) were plated in 24-well plates in medium containing 10% steroid-depleted fetal calf serum [33] and suramin 300 μg/ml. Increasing concentrations of testosterone were then added. 24 h later the cells were pulsed and incorporated radioactivity precipitated and counted as reported above. *Points*: mean values of 2 experiments each in duplicate; the SEM $<15\%$.

through a lowering of circulating adrenal androgen levels.

Sixteen patients with metastatic adrenocortical carcinoma have been treated with suramin, either by bolus injection or continuous infusion, and are evaluable for response [16]. Given suramin's prolonged half-life, the common endpoint of these two different dosing schedules was the achievement of a plasma suramin level >150 μg/ml. Of these patients, 2 achieved a partial response, of 2 and 6 months duration, defined as a 50% decrease in the summed products of the perpendicular diameters of all evaluable lesions. An additional 2 patients had a minor response to suramin treatment and 5 remained with stable disease for periods ranging from 3 to 10 months. The remaining 7 patients progressed on suramin therapy.

Thus far, 35 patients with metastatic prostate cancer refractory to at least one conventional hormonal manipulation have been treated with suramin by continuous infusion and are available for interim analysis [29]. Fifteen of these patients had metastatic disease involving one or more soft tissue sites and 3 of these (20%) have manifested complete disappearance of their measureable soft tissue disease with suramin therapy. Soft tissue sites of tumor involvement were lymph nodes and biopsy-proven skin nodules. With regard to the bone involvement by prostate cancer, thus far only 3 (8.6%) of the 35 patients have demonstrated some improvement in bone scan abnormalities, and in each case this became manifest only after at least 9 months of treatment. An additional 17 patients have manifested either equivocal or no changes in their bone scans with suramin therapy, and the remaining 15 patients have progressed. Prostatic specific antigen (PSA) is a tumor marker whose serum concentration is useful in monitoring response and recurrence after therapy [31]. In addition, serum PSA levels appear to correlate with estimated tumor volume at least in patients with newly diagnosed prostate cancer. Serial measurements of PSA levels have been performed in 32 of these patients in the course of suramin therapy and 7 (21.9%) have had normalization of their levels. However, only in 4 of these 7 patients has the duration of PSA normalization thus far been >3 months. In each of the prostate cancer patients treated with suramin who eventually manifested some degree of tumor response (disappearance of soft tissue disease, normalization of serum PSA levels or improvement in bone scan), evidence of this nearly always appeared in the course of the first cycle of therapy (i.e. during the first 10 weeks

on study), either with shrinkage of soft tissue disease or a significant deline in PSA levels.

Seven patients with heavily pretreated nodular lymphoma have been treated with suramin and are available for response [30]. In each instance, suramin therapy was instituted in the setting of objective disease progression and when an indication for therapy existed (i.e. progressive thrombocytopenia, impending renal obstruction, worsening B symptoms etc.). Four of these seven patients achieved a partial response with marked shrinkage or disappearance of peripheral and central adenopathy. In addition, improvement of hematologic parameters, disappearance of both B symptoms and biopsy-proven malignant skin lesions were documented. In each case however, scant bone marrow involvement with lymphoma persisted.

SURAMIN-RELATED TOXICITIES

The side-effects resulting from suramin administration to cancer patients can be significant, and are the subject of recent reviews [4, 5]. Perhaps most significant has been the development of a severe polyradiculoneuropathy in 4 of the first 38 cancer patients treated with suramin [32]. In this treatment group, no correlation with total suramin dose administered nor duration of therapy could be made. However, each of these 4 patients had achieved elevated maximum steady-state plasma suramin levels during treatment (i.e. $>386\ \mu g/ml$). As a result of this association, our group placed a major emphasis on continued pharmacokinetic monitoring during suramin treatment, and retreatment at 2-month intervals. As a consequence, the incidence of this severe surmain-related toxicity has declined markedly.

Additional side-effects associated with suramin administration include: rash, infections (often related to the presence of an indwelling catheter), vortex keratopathy, coagulopathy, thrombocytopenia, renal function abnormalities and a fatigue–malaise syndrome.

CONCLUSION

Suramin, in view of its growth factor and enzyme-binding properties, represents in many respects the prototype of a new class of antitumor compounds. Although the objective response rate in our preliminary studies with this compound in adrenocortical carcinoma and hormone resistant prostate cancer are relatively modest, that achieved in heavily pretreated nodular lymphomas is promising and merits further study. A better understanding of suramin's postulated antitumor mechanisms and improvement of the dosing schedule in treating cancer patients are the major focuses of our research group. In addition, efforts to develop structurally related compounds with potentially less toxicity are underway.

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