Suramin: A Novel Antineoplastic Agent with Multiple Potential Mechanisms of Action

C. A. Stein

Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, New York 10032

In the June 1992 edition of the Journal of Clinical Oncology, Myers et al. (1) reported on 38 cases of metastatic hormone refractory prostate cancer treated with suramin, a bis-polysulfonated naphthylurea. In the accompanying editorial, Pinedo and van Rijswijk (2) state that "there can be little doubt that suramin represents an important new group of anticancer agents." In fact, the recent encouraging results with suramin as an antineoplastic agent have occurred as a result of decades of patient groundwork, followed by a few key observations in patients with acquired immunodeficiency syndrome being treated with suramin as part of a clinical therapeutic trial. The drug is highly unusual, both in its biological properties and in the story of its development as an antineoplastic agent. However, this story may have some valuable lessons to teach with respect to the identification of novel agents with potential antineoplastic activity.

The story of suramin begins with the discoveries of Paul Ehrlich, who had at his disposal the organic dyestuffs produced by the German chemical industry from the coal tar wastes of Ruhr steel production (3). He was faced with the problem of devising a treatment for African trypanosomiasis, which was ravaging cattle herds and human populations in what was then German East Africa. In the first few years of the 20th century Ehrlich and Shiga discovered that certain bis-polysulfonated naphthalene azo dyes, e.g., trypan red and trypan blue, were active antitrypanosomal agents. However, the activity of the dye compounds was not sufficient for veterinary use, and worse, the meat of the cattle became discolored after treatment. On the basis of this early work, the Bayer company, probably responding to a military request, in 1909, 14 years after Ehrlich's discovery, began to develop a new, more reactive dye, suramin (4). Widespread clinical use followed; the dose was 1 g daily for a week.

Widespread, world-wide use followed; the dose was 1 g weekly for 6 weeks. By 1947, it was appreciated that a cohort of Zairean patients with trypanosomiasis were also being cured of coinfection with onchocerciasis. Although more recently supplanted by other agents, suramin, by virtue of its cost, is still being used for its original indication in poorer areas of the world. Only 4 years ago, a traveler to the southern Sudan remarked to me on the use of suramin as an antiparasitic agent in the native population.

Over the next several decades, an important body of literature had been compiled which was summarized in a review compiled by Hawking (6). He noted that the drug could bind to and inhibit the function of a wide variety of enzymes and proteins, although he could detect no underlying unifying themes. These enzymes included hyaluronidase, fumarase, urease, hexokinase, Na⁺-K⁺ ATPase and RNA polymerase. Suramin was also a potent in vitro anticoagulant, and an in vivo teratogen. Its clinical pharmacology and toxicology were, in retrospect, not understood well.

In 1979, DeClerq (7) appreciated that suramin could inhibit the activity of the reverse transcriptase found in RNA tumor viruses, such as the murine leukemia, Moloney sarcoma, and avian myeloblastosis viruses. For the latter, at least, the inhibition was competitive with (rA)n-oligodeoxythymidylate. These data were subsequently confirmed by Basu and Modak (8). By 1984, Mitsuwa, working in Broder's laboratory at the NCI, had noted that suramin could protect uninfected T-cells from the cytopathic effect of HIV-1 (9); it was presumed that at the time that this was a consequence of reverse transcriptase inhibition. Because of these encouraging results and to due to the dearth of active therapies for patients afflicted with AIDS, the drug entered clinical trials at several centers, including the NCI and the University of Southern California (10–13).

In the fall of 1985, we began to care for patient B. H., who was infected with HIV-1 and had multiple cutaneous lesions of Kaposi's sarcoma. He had been receiving suramin therapy for approximately 1 year. B. H. was also suffering from Addison's disease; the older toxicology literature indicated that adrenocortical atrophy had indeed been reported as early as 1937 (14) as a toxicity of suramin administration to guinea pigs. Treatment of B. H. with appropriate glucocorticoids and mineralocorticoids led to rapid relief of his symptoms, but his treatment with suramin was halted. (The Kaposi's sarcoma then underwent explosive growth, culminating in the patient's demise within 8 months from internal bleeding secondary to visceral disease.) Suramin was eventually deemed to be ineffective treatment for HIV-1 disease; improvement was noted neither in immunological function nor in clinical status. Significant toxicity was also discovered. However, because of the lack of understanding of the clinical pharmacology of this agent, it was not at the time appreciated that due to the dosing schedule then used, toxicity could have been related to chronically elevated maximum achieved plasma suramin levels.

For HIV patients who was not individualized; plasma suramin levels were batch-determined, sometimes months after patients were dosed. Similarly, no correlations were or could be drawn between the plasma suramin area under the concentration curve and clinical or immunological response. Furthermore, at the time of these trials (1985–1987), individualized patient dosing by means of the Bayesian pharmacokinetic parameter value estimator, as developed by Lieberman et al. at the NCI (15) and Sher et al. (16) at the University of Maryland, was
not available. This method, discussed below, has effectively eliminated the majority of the Grade 3-4 toxicities associated with suramin therapy in the prostate cancer population and may allow suramin to be delivered to the HIV-infected population with reduced toxicity. Furthermore, at the time of the HIV trials, other potential mechanisms of anti-HIV activity [e.g., inhibition of PKC kinase activity (17, 18)] were also not appreciated, nor was the potential of suramin for direct antineoplastic activity. For example, it is now recognized that suramin is capable of binding to both K-FGF and basic FGF (19) and can block the binding of both to their respective receptors. This effect has been used to advantage by Moscatelli and Quarto (20) to inhibit the growth and reverse the phenotype of fibroblasts transformed by these oncogenes. These data suggest that suramin may be active in HIV-related Kaposis’s sarcoma, and a human trial is being planned, in which the suramin dosing will be adaptively controlled (16) to reduce toxicity.

The human adrenolytic properties of suramin were confirmed in the HIV trial of Levine et al. (10). Historically, mitotane, an active agent in adrenocortical cancer, had been discovered in a similar manner. Dogs that had been fed the insecticide DDT became Addisonian, and the active toxic principle was traced to the contaminant mitotane. Was it possible that suramin would behave similarly and would show activity in adrenocortical cancer? By this time (1985) it had also been recognized that suramin could block the binding of at least one growth factor (PDGF) to its receptor (21). Given the role of substances such as PDGF in the promotion of tumor cell growth, the possibility existed that suramin had potential application as an antineoplastic agent. The high affinity of suramin for a wide variety of biological targets was not daunting; it was, if anything, encouraging. It was, and remains, unclear if any active anticancer agent acts only by a single mechanism or at a single site, and a preponderance of evidence indicates that resistance would develop rapidly if that were the case. Might not suramin, then, interrupt critical pathways in tumor cells at lower concentration than in normal cells? Thus it was that in 1985-1986, when these, in retrospect, naive thoughts, that myself and C. E. Myers, with clinical assistance from R. LaRocca, brazenly plunged forward into clinical trials with suramin in malignant disease.

Clinical Trials

Cancer of the Prostate. Hormone refractory metastatic prostate cancer patients have an average survival time of 30 weeks (22), and the aggregate response to current chemotherapeutic agents is 7% (23). The first patient with hormone refractory metastatic prostate cancer placed on suramin was R. M., who was entered as part of a general phase II trial. This patient, as were the 37 others in the NCI trial (1), were treated by the continuous infusion schedule. Twenty-one patients had bone only disease, and 17 had measurable masses. Three of the 17 had complete resolution of measurable disease for 4, 5, and 11 months. Three additional patients had a >50% radiographic reduction of their measurable disease.

There was no radiographic resolution of bone lesions. However, 21 patients experienced a decline in serum PSA of >50%, and 13 experienced a decrease in PSA of >75%. In this latter group of patients, the survival probability at 1 year was 85%; while in all other groups, it was 20%. Indeed, the median survival for the patients experiencing the >75% drop in PSA has presently not been reached at 3.5 years. Pretreatment level of PSA was closely correlated with PSA response, 7 of 10 patients with pretreatment with PSA <100 ng/ml experienced a >75% decline with suramin treatment, but only 6 of 28 patients with PSA >100 ng/ml experienced the same decline. The extent of pain relief was striking; 71% (15 of 21) of patients were able to reduce by one-half or eliminate their use of narcotic analgesics. A similar trial of suramin in metastatic hormone refractory prostate cancer, carried out by Eisenberger et al.3 has confirmed the major features of the NCI trial and will be in press shortly.

However, the interpretation of the pain relief data is confounded by the cotreatment of patients with hydrocortisone, required due to the clinical adrenolytic effects of suramin. Hydrocortisone, alone, may cause significant pain relief (24) and may cause transient decreases in PSA. Nevertheless, it is difficult to believe that the apparent prolongation of life in that cohort of patients that experiences a >75% decline in PSA with suramin treatment (median survival not reached at 3.5 years) is due merely to the hydrocortisone. Moreover, this apparent prolongation seems to confound the idea that suramin is only diminishing PSA production by the tumor but is doing little or nothing to prevent tumor growth. However, the characteristics of patients with metastatic prostate cancer accrued onto the several ongoing clinical trials with suramin must be examined carefully so that proper comparisons among trials can be made. The data from the NCI trial clearly indicate that the likelihood of achieving the >75% decline in PSA is inversely related to the PSA at presentation; i.e., the higher the PSA, the worse is the “response rate.” Differences in response rates between institutions may correlate with PSA levels at presentation, which in turn may be a reflection of the biological aggressiveness of the tumor (as underscored by the tumor Gleason grade), the tumor bulk, or other, as yet undetermined factors. Implicitly, then, if the NCI data are confirmed, a case could be made for the earliest possible diagnosis of recurrent prostate cancer, when presumably it would be the most treatable by suramin. This might then be a major stimulus to ongoing studies using, e.g., monoclonal antibodies as imaging agents for microscopic disease, and to the further development of “supersensitive” assays for PSA. The prospect of early diagnosis coupled with effective therapy for this refractory disease would indeed be an exciting one for the clinical oncologist, but a great deal of work lies ahead to validate these concepts.

Cancer of the Adrenal Cortex. The original pilot trial with suramin as an antineoplastic agent was conducted in patients with adrenocortical cancer (25, 26). Patients were treated either with a weekly i.v. bolus or by a constant infusion until the plasma suramin level reached 300 µg/ml. Seventeen patients were available for response; 11 of the 17 had previously been treated with mitotane. Two achieved a partial response, 2 had a minor response, and 5 had stable disease for 3–10 months. The remainder progressed. One of seven

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3 M. Eisenberger et al., personal communication.
who had abnormally elevated plasma steroid hormone levels had a 50% decline in multiple steroid precursors. An additional patient, who did not have radiographically demonstrable disease but who did have elevated plasma aldosterone levels, hypertension, and hyperkalemia, became normotensive and normokalemic after suramin treatment. Responses were observed predominantly in pulmonary metastases. On the continuous infusion schedule used in these trials, the activity of suramin in adrenocortical cancer was probably not greater than that of mitotane, while toxicity was severe. The use of suramin as a therapeutic agent in this tumor must be reevaluated with the newer dosing schedules (see below).

**Lymphoma.** Suramin is active in lymphoid cell lines (27) and in chemotherapy resistant follicular lymphoma as well. Ten patients (28) (nine evaluable, seven with follicular mixed, and three with follicular, small cleaved cell lymphomas) received suramin. Each had failed from one to six other regimens. Five of the nine achieved partial remissions. Sites of response included peripheral and central nodes, spleen, skin, bilateral pleural effusions, and bone marrow, and some of these responses were quite dramatic and remarkable for a single agent. Response duration varied from 3 to 9 months (median, 8 months). Follow-up of these initial interesting results is desperately needed, particularly given recent increases in the incidence of the disease. No clinical information exists on the use of suramin in large-cell lymphoma.

**Other Tumors.** Suramin did not have activity against metastatic renal cell cancer (29), except in one patient with Staufer syndrome. Interestingly, a renal cell line grown from another patient with metastatic renal cell cancer and Staufer syndrome was also sensitive to the inhibitory effects of suramin (30). A preliminary report showed no evidence of response in 10 patients with advanced, platinum resistant ovarian cancer either (31), in spite of the recent demonstration of activity in ovarian cancer cell lines (32). However, prior to suramin treatment, 6 of 10 patients had received 3 or more chemotherapeutic regimens. Of great interest would be a trial of i.p. suramin in refractory ovarian cancer. It is most likely that very high levels of suramin can be attained with little or no systemic toxicity. Furthermore, malignant cells can frequently be withdrawn via paracentesis and treated prior to therapy; this may guide or help to individualize drug dosing. Phase I trials are eagerly awaited.

A similar logic may be applicable to carcinoma in situ of the bladder. Extremely high levels of suramin may be obtained in this hollow viscus by direct instillation with little local and no systemic toxicity.

**Clinical Toxicity of Suramin.** This problem has been extensively reviewed by Stein et al. (33). Major toxicities in the NCI prostate cancer study were: hematological (anemia, lymphopenia, thrombocytopenia); renal; hepatic; hyperglycemia; skin rash; fatigue; fever; nausea; hypophosphatemia; adrenal insufficiency. Sixteen of 38 patients experience culture documented bacterial infections. Seventy-nine % of patients experienced NCI Cancer Treatment Evaluation Program (CTEP) grade 1-2 peripheral sensory neuropathy. The coagulopathy (34) and motor neuropathy [Guillain-Barré-like syndrome (35)] seen in the earlier trials could be prevented by careful monitoring of plasma suramin levels.

**Pharmacology.** Early work by Collins et al. (36) demonstrated that suramin was highly (99.7%) bound to plasma proteins and that the total body clearance is very low (0.41 ml/min). The rate of total body clearance, predominantly via the kidney, displays little variation between individuals (coefficient of variation, 15%). The terminal half-life of the drug is 40–50 days. However, when the drug was given via continuous infusion, a significant interpatient variability in plasma suramin levels was noted (16). The elimination of suramin from plasma, at least during the first few weeks of drug administration, is dominated by drug distributional clearance to organ sites, rather than renal clearance. Plasma clearance has been modeled by a two compartment linear open process in which drug elimination occurs only from the central compartment. However, the variability in rate of drug movement from the central to peripheral compartments (e.g., liver, spleen, kidney) is high, explaining the interpatient variability in plasma suramin level. This variability created a dosing problem because of the problem of suramin induced neurotoxicity (35). The neuropathy, which resembles the Guillain-Barré syndrome, occurs at plasma suramin levels exceeding 350 µg/ml and can be characterized by profound motor weakness culminating in ventilatory failure. This suggested the need for individualized patient dosing based on measurements of pharmacokinetic parameters and led to the development of the adaptive control strategy for suramin dosing (15, 16, 37). In practice, prediction of plasma suramin levels were obtained on a minicomputer (ADAPT II software) by using the Bayesian algorithm, knowing the individual dosing history, and the plasma suramin levels at any given time. Then, the individual bolus dose was adjusted so that the desired plasma suramin level was achieved at any future time point. The adaptive control method of drug dosing ensured that plasma suramin levels did not rise above 350 µg/ml and eliminated the toxicity of suramin induced Guillain-Barré syndrome as well as ameliorating many others. However, this method is quite cumbersome and may severely limit the widespread clinical application of this drug. It has now become fairly clear that this stringent pharmacological monitoring may not be necessary. Several clinical trials are now ongoing that eliminate the adaptive control algorithm entirely and replace it with a fixed bolus schedule that mimics the computer generated dosing scheme. Transient, extremely high (>400 µg/ml) plasma suramin levels may be reached after the initial bolus, but systemic toxicity appears to be minor. The establishment of a universally agreed upon dosing schedule will certainly hasten progress in resolving many issues surrounding the use of this agent. For example, should patients be treated to toxicity, or are “rest periods” between “cycles” more productive of higher response rates? What is the relationship between administered drug quantity, toxicity, and response? What is the minimum effective plasma level of suramin, and for what length of time must it be maintained?

**Potential Mechanisms of the Antitumor Effect**

**Inhibition of Growth Factor-Receptor Binding.** Over the past decade, it has become increasingly clear that suramin is, among its other capabilities, able to function as a competitor of glycosaminoglycan-binding to a variety of targets. GAGs, while used in connective tissue and basement membrane as scaffolding material, are also the substances that bind extracellular growth factors. Binding is competitive with respect to heparin, and perhaps with respect to suramin as well.

The molecular weight of suramin is 1429, its structure is shown in Fig. 1. The six sulfonate groups are completely ionized at physiological pH. A common structural motif underlies the ability of suramin to bind to the heparin binding growth factors. This has been demonstrated by Baird et al. (38) who have found that two distinct, highly basic domains of 10-15 amino acids in the bFGF molecule have both heparin binding and growth stimulating activity. In fact, bFGF crystallizes with a sulfate ion at what has been suggested is the heparin binding site (39). Rigidity of the ring bridging backbone is conferred by the presence of rotationally restricted polyamide and urea groups. These may lower the dissociation constant by diminishing the entropic component of binding to the growth factor target. However, detailed

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4 N. Vogelzang, personal communication.
kinetic and equilibrium studies on the binding of suramin to heparin binding growth factors have yet to appear.

Hosang et al. (40) was able to demonstrate that suramin could prevent the binding of PDGF to its cell surface receptors on fibroblasts. This was correlated with decreased cellular proliferation. On the other hand, at least in glioma cell lines (41), blockade of PDGF-receptor binding by suramin is not necessarily predictive of growth inhibition. In addition, at least one cell line not possessing measurable PDGF receptors was still inhibited by suramin treatment, implying the drug may be active by more than one mechanism. Similar data have been generated by Powis et al. (42) who used polyanionic growth factor binding organic dyes that are membrane impermeant. In Swiss 3T3 fibroblasts, blockade of growth factor-receptor binding could be demonstrated, but not cellular growth inhibition.

A series of interesting experiments have been performed in cells transformed by the simian sarcoma virus (43-45). These cells express the Mr 28,000 protein product of the v-sis oncogene, which is closely related to PDGF. It is frequently the case that the PDGF receptors, especially in simian sarcoma virus transformed cells, are not expressed in their mature form on the cell surface, but rather that PDGF receptor binding occurs internally. Suramin, however, can block these internal autocrine loops as well as the external loops (45). Presumably because of this ability to dissociate bound PDGF from its receptors, reversion to normal of the simian sarcoma virus transformed phenotype in fibroblasts has been observed (46).

Suramin, because of its charge, is membrane impermeant. Therefore, it must be actively transported into cells, and it is virtually certain that cells will exert control over the rate of suramin internalization. The factors regulating this rate are currently unknown but may be central to the ability of suramin to behave as a cytotoxic agent. The data presented above (46) provide evidence that suramin, which is internalized probably predominantly via the processes of adsorptive endocytosis and/or fluid phase endocytosis or pinocytosis,5 is accessible to extralysosomal intracellular compartments. This is an important finding, given the ability which most cells appear to have to exclude some polyanions (e.g., trypan blue) which have structures that are closely related to that of suramin. These data may imply that suramin, after internalization, can also penetrate the endocytic vacuole, but how this occurs is not known.

Sjolund and Thyberg (47) examined the effects of suramin in primary cultures of smooth muscle cells. These cells produce PDGF which promotes growth in an autocrine manner. Treatment with suramin led to morphological transition from a normal contractile state to a state in which myofilaments were lost and the amount of rough endoplasmic reticulum increased. This is the state of the smooth muscle cell frequently seen in atheromas, and it is thus interesting to speculate on the potentiation of these lesions in patients receiving long-term suramin therapy for nonmalignant disease.

PDGF is a heparin binding growth factor. There are many other growth factors, some of which are known to be heparin binding, with which suramin appears to be able to interact. For example, Coffey et al. (48) examined the effects of suramin on the mouse embryo AKR-2B, line, whose growth is sensitive to TGF-ß and bFGF, and demonstrated growth cessation and diminished titrated thymidine incorporation in the presence of 100 μM suramin. On the other hand, TGF-ß acts as a growth inhibitor in renal cell carcinoma lines. In the presence of 300 μg/ml suramin, growth was restored almost to the control level seen in the absence of drug (49).

Another important group of heparin binding growth factors are the family of fibroblast growth factors (FGF). The members of this group include acidic and basic FGF, int-2, K-FGF/hst, FGF 5, and FGF 6. These proteins all bear significant sequence homologies, and some members of this group, including bFGF, are potent stimulators of angiogenesis and neovascularization. For example, bFGF can stimulate endothelial cell proliferation, as well as migration and invasiveness (50). In addition, bFGF stimulates the growth of osteoblasts and promotes their formation of calcium phosphate crystals in culture. High levels of expression have been reported both in benign prostatic hyperplasia and in prostate carcinoma (51-55). bFGF, because it does not bear the appropriate leader sequence, is not secreted via the classical secretory pathway and is thought to exert its effects via an internal autocrine loop, although this view has recently been challenged (50). Extracellular bFGF, on the other hand, is never found free but is complexed with glycosaminoglycans (e.g., heparan sulfate) in basement membranes. In this context, suramin may behave as a GAG analogue, inasmuch as it binds to bFGF and blocks its binding to a cellular receptor.

Inhibition by suramin of the bFGF effects on growth and DNA synthesis were first observed by Coffey et al. (48) in the mouse AKR-2B line and confirmed by others (19). The concentration of suramin required for maximal effect was well within clinically achievable and tolerable plasma suramin levels (300 μg/ml). The initial cellular response of bFGF, the phosphorylation of a Mr 90,000 protein, was blocked by suramin (56, 57). Moscattelli and Quarto (20), in 3T3 fibroblasts, reverted the bFGF transformed phenotype to normal with suramin (1 μM). Similar effects were also observed in cells transformed by K-FGF, which appears to use the same receptor as bFGF (58).

The ability of suramin to block the binding of bFGF to its receptor suggests that the drug might be an antiangiogenic agent. Other data that suggest this possibility relate to the ability of suramin to bind to a newly purified heparin binding growth factor, vasculotropin/vascular endothelial cell growth factor, a member of the PDGF family. This protein stimulates the growth of human umbilical vein endothelial cells and induces capillary cord formation. It can be dissociated from its receptor, to which it binds with picomolar Kₐ by suramin (59-61). Indeed, Wilks et al. (62) and Gagliardi et al. (63) have recently shown that suramin can replace heparin, in combination with steroids in the chick embryo chorioallantoic membrane assay. Depending on the assay system used, suramin may be angiostatic in the absence of added steroids. It is unknown at the current time whether inhibition of the neovascularization of solid tumors may related to the observed antineoplastic effects of this agent.

Other Growth Factors. Suramin also appears to be capable of inhibiting the binding of IGF-1 (pl 9.2) to osteosarcoma cells in culture (64), but it is uncertain whether the effect occurs at the level of the growth factor or of its receptor. This results in a dramatic decrease in cellular growth after 6 days. In nude mice, however, the growth of osteosarcoma xenografts was markedly inhibited after transplantation, and the effect persisted, during weekly suramin treatment, for up to 100 days posttransplantation (65). IGF-II, while sharing 60% homology with IGF-1, is slightly acidic (pl 6.5), but is probably heparin binding. It functions as an autocrine growth factor in the human rhabdomyosarcoma line RD and is at least partially displaced from its receptor by suramin. This appeared to correlate well with inhibition of cellular proliferation (66). Scatter factor, a heparin binding protein produced by nondifferentiating keratinocytes, has, in one of its subunits, amino terminal sequence homology with the smaller subunit of human hepatocyte growth factor. Scatter factor causes dispersal of epithelial cell colonies, but this effect was blocked at 250 μg/ml of suramin (67).

Epidermal growth factor binds to heparin with only an extremely low affinity and in general is less sensitive to suramin than are growth factors that demonstrate high affinity heparin binding (47). [In several...
urothelial cell lines that are growth inhibited by suramin (e.g., T24, where IC50 is 36 μM, and HT 1376), however, EGF binding to its receptor is significantly more sensitive to inhibition by suramin than it is in AKR-2B cells (68)). In the A431 squamous carcinoma line, suramin was able to indirectly activate the EGFR by causing the release into the medium of TGF-α, which could then bind to EGFR, its natural receptor (69). The process of release by suramin of a mature, secreted, but cell surface bound growth factor has also been observed for the non-heparin binding murine protein, Wnt-1 (formerly int-1 (70, 71)).

On the other hand, data concerning tumor cell growth stimulation by suramin was obtained by Olivier et al. (72), who noted low concentration (50–125 μg/ml) growth stimulation in 16 of 25 lines studied, including melanoma and osteosarcoma lines. A weak correlation existed between the number of EGFR per cell and the corresponding suramin IC50 values, but many lines without EGFR were also growth stimulated. The stimulation of tumor growth at low suramin concentration is of particularly great concern clinically, given the reliance of the older trials on continuous infusional methods of drug delivery. It strongly suggests that intermittent bolus dosing (which rapidly raises the plasma suramin level to the maximum tolerated dose), either by fixed dose or by adaptive control monitoring as described above, should be the preferred method of treatment.

Suramin is also capable of inhibiting the binding of IL-2 to both the IL-2α and IL-2β receptors (73). Subsequently, IL-2 induced receptor phosphorylation and IL-2 induced proliferation are also blocked, with cells becoming unable to enter S phase. In some cell lines, the effects of suramin treatment could be entirely overcome by addition of exogenous IL-2. However, relatively high levels of suramin (1 mg/ml) are required for these effects, and this is much higher than what can be tolerated in human beings. IL-2 contains a nucleotide binding site (74) which may well be the suramin binding site as well (see below), and it is possible that this interaction causes a conformational change in the protein that prevents receptor binding. On the other hand, the binding of human TNF-α to cell surface receptors on K562 cells was blocked by suramin with a much lower IC50 (175 μM) (75). The cytotoxic effects of human TNF-α in LM cells were also significantly ablated by suramin (<200 μg/ml). It is interesting to speculate that perhaps the improved sense of well-being experienced by many cancer patients within a short time of commencing suramin therapy relates to a suramin induced decrease in the effective activity of cachectin/tumor necrosis factor. Furthermore, high circulating levels of TNF-α have been correlated with poor response to treatment with recombinant human α-interferon in chronic myelogenous leukemia, with severity of graft versus host disease, and with the development of venoocclusive disease and sepsis. The ability of suramin to block the binding of TNF-α to its receptor suggests additional clinical potential for this drug.

Suramin can also inhibit the binding of transferrin to its receptor (76). This may result in decreased cell growth, particularly in prostate cancer metastatic to the bone (77), which seems to be highly dependent on transferrin for growth. Apolipoprotein B, the heparin binding protein component of LDL, a major cholesterol binding particle, is, like transferrin, also internalized via the process of receptor mediated endocytosis. LDL, too, is blocked from binding to its receptor by suramin (78). Furthermore, suramin can also inhibit other stages in the process of receptor mediated endocytosis. This includes inhibition of the vacuolar H+ -ATPase (79), the protein that acidifies organelles of the vacuolar system, including the endosome. Lack of endosomal acidification may result in lack of receptor-ligand dissociation and a subsequent decrease in receptor (e.g., transferrin receptor or LDL receptor) recycling back to the cell surface. Furthermore, we (80) have shown that in HL60 cells suramin, in a manner similar to that of other inhibitors of protein kinase C activity, including H7, dimethyl sulfoxide, staurosporine, and phosphorothioate oligodeoxynucleotides, also block internalization of fluid phase markers via the process of pinocytosis, or fluid phase endocytosis. Free suramin, as well as suramin bound transferrin, LDL, or albumin may enter cells by this process. The inhibition of fluid phase endocytosis further blocks the ability of rapidly dividing cells to obtain appropriate raw materials and may be an additional growth suppressive mechanism.

**Inhibition of Other Heparin Binding Proteins: Inhibition of Glycosaminoglycan Metabolism.** In plasma, suramin is >99% protein bound, mostly to albumin. The affinity is low [Kd approximately 3 3 10-6 M (81)]. It is likely that hydrophobic interactions between suramin and albumin (82) contribute to the stability of the complex. After internalization, suramin is found in lysosomes (6, 83-85), bound, perhaps, to lysosomal hydrolases, the functions of which it differentially inhibits. Brady and Constantopolous and their coworkers (86-90) have studied in detail the effects of suramin of those lysosomal hydrolases which are responsible for GAG catabolism. They recognized that genetic defects in lysosomal enzymes are responsible for a set of illnesses known as the lysosomal storage diseases, or mucopolysaccharidoses. In children thus afflicted, accumulation of uncatabolized GAGs, such as the sulfates of heparan, dermatan, and chondroitin, in organs such as the brain, liver, spleen, and kidneys results in functional compromise. In experimental animals (91), the enzyme activities of the lysosomal enzymes iduronate sulfatase, β-glucuronidase, and hyaluronidase were consistently decreased after suramin treatment. This resulted from direct, although non-competitive (with respect to GAG substrate), inhibition of these enzymes. Purified rat liver iduronate sulfatase was 100% inhibited at a suramin concentration of 50 μM. β-Glucuronidase was not as sensitive. However, the sensitivity of iduronate sulfatase to inhibition by suramin interferes critically with net GAG catabolism. This enzyme is required for removal of the sulfate group on position 2 of the iduronic acid residues of heparan and dermatan sulfates. If 2-O desulfation is inhibited, then the subsequent catabolic step, cleavage of the terminal glycosidase bond by α-L-iduronidase, will also be inhibited. Thus highly sulfated, noncatabolized GAGs will accumulate in lysosomes and, eventually, in the blood of patients. In effect, suramin treatment induces an acquired mucopolysaccharidosis syndrome that is reminiscent of Hunter disease, or mucopolysaccharidosis II. The picture is complicated by the fact that β-glucuronidase can be inhibited by suramin. This enzyme is required for cleavage of the β-glucuronyl linkages found in chondroitin 4 and 6 sulfates, hyaluronic acid, and dermatan and heparan sulfates. Inhibition of this enzyme increases plasma levels of all of these GAGs and resembles mucopolysaccharidosis VII, also known as Hurler’s disease. [Suramin will also cause increases in levels of the gangliosides Gm2, Gm3, and D2 (91, 92) by inhibition of sialidases.] The uncatabolized GAGs accumulate in the bloodstream, where they are weakly anticoagulating (93); in the cornea of the eye (94), where they cause vortex keratopathy; and potentially in other organs as well. However, it is also possible that these accumulated GAGs may have antineoplastic activity in their own right, by virtue of their ability to bind and sequester growth and angiogenic factors, to compete with extracellular matrix GAGs for heparanases secreted by metastasizing tumors, and possibly to serve as regulators of nuclear function, all of which appear to be properties of suramin as well.

The ability of suramin to inhibit lysosomal enzymes may have additional clinical toxicities. For example, hyperglycemia is now a well-recognized toxicity of clinical suramin treatment. In the mouse, a pancreatic β-islet lysosomal enzyme, acid amyloglucosidase, appears to affect levels of insulin secretion in mice (95-97). This enzyme was inhibited in a dose dependent fashion by suramin. The drug
also blocked the insulin response of the sulfonylurea secretogogue glibenclamide, which itself appears to act by blocking the ATP sensitive K+ channel (98). Data of these kinds must be considered in planning the management of clinical suramin toxicity.

Other Mechanisms of Antitumor Inhibition. Mammalian DNA polymerases are also heparin binding proteins, and suramin is able to inhibit their function as well. The drug behaves like a double stranded oligonucleotide analogue, exemplified by its ability to competitively inhibit DNA polymerase α (a replicative polymerase) with respect to template-primer binding (99–101). The DNA primase activity associated with DNA polymerase α was also inhibited by suramin, but inhibition was competitive with respect to dGTP. Polymerases β (the DNA repair polymerase) and γ, on the other hand, were somewhat more resistant to suramin treatment (27). Polymerase δ, which may be the leading strand DNA polymerase in eukaryotic cells, was inhibited by suramin with IC50 = 36 μM.

The ability of suramin to compete with deoxynucleotide triphosphates, especially ATP, for their binding sites may play a critical role in the activity and toxicity of suramin. In work by Calcaterra et al. (102), the ATPase activity of submitochondrial particles was inhibited by suramin, and the inhibition was also competitive with respect to ATP binding. Indeed, Rago et al. (103) have suggested that disruption of ATP generation by the mitochondrion may be one of the major mechanisms of cellular cytotoxicity in the DU145 prostate carcinoma cell line. Supporting this view are data (102) which demonstrate that suramin inhibits the submitochondrial adenine nucleotide translocase complex, which functions to transport AMP and ADP across the mitochondrial membrane. Other examples of competition with deoxynucleotide triphosphates are also known (104).

Of potentially great significance are recent observations that suramin has been shown to competitively inhibit the phosphorylating ability of PKC (isoforms I, II, and III, encoded by the γ, β, and α genes, respectively) with respect to ATP (17, 18). Moreover, the inhibition demonstrated a degree of isoform specificity, with the order of inhibition being I > II = III. Phorbol ester-induced PKC activation was also blocked in the presence of suramin. Furthermore, at low suramin concentration (<50 μM), in the presence of calcium, but in the absence of required lipid cofactor stimulation, suramin actually stimulated PKC activity by as much as 300%. Stimulation of activity was also isoform dependent, with I > II > III. We have recently shown that these biphasic concentration effects on PKC phosphorylating ability are not confined to suramin alone but also occur with other bis(polyamionic) compounds containing hydrophobic bridges. However, PKC is a critical intracellular enzyme involved in many transmembrane signaling events and may also be involved in tumor promotion (105). The unusual biphasic response to suramin and other bis(polyamions) may be a critical factor in determining tumor response to alternative drug dosing schedules. In addition, the complexity of the in vitro actions of suramin, as exemplified by its interactions with PKC, tend to invalidate overly simplistic explanations for its in vivo activity. For example, it has been assumed that the cytotoxic effects of suramin against HIV-1 are due to the ability of the drug to inhibit the HIV-1 reverse transcriptase. However, Fields et al. (106) have recently shown that the PKC inhibitor H7 (an isoquinoline sulfonamide) also reduces HIV-1 infectivity, possibly by blocking HIV-1 induced phosphorylation of CD4 by PKC. Suramin, which at a higher concentration is a potent PKC inhibitor, may inhibit HIV-1 replication via this cytoprotective pathway.

Alteration of signal transduction pathways by suramin is not limited to its effects on PKC activity. Suramin can down-regulate both diacylglycerol kinase and phosphoinositide kinase activity (107). In the latter case, this may lead to decreased levels of phosphatidylinositol and phosphatidylinositides. This may decouple the cellular proliferative response from a mitogenic signal derived, e.g., from growth factor stimulation. Related to this, Seewald et al. (108) have observed that suramin also appears to cause a decrease in the intracellular calcium response to growth factor binding; this may be in fact due to inhibition of phosphoinositide synthesis (see below) and may augment suppression of PKC activity, which is calcium dependent. These data suggest that suramin, like doxorubicin, may be an example of a self-synergistic agent, i.e., one which can act on sequential steps in an in-series pathway.

Diacylglycerol kinase, on the other hand, is a substrate for PKC, and an enzyme necessary for the resynthesis of phosphoinositides. It is also the enzyme that catalyzes the formation of phosphatic acid, a promoter of DNA synthesis and cellular growth (109). It appears to do this, in part, by activating membrane bound G-proteins, which are also sensitive to inhibition, in vivo by suramin (110).

An additional mechanism of action of suramin may involve inhibition of nuclear DNA topoisomerase II (111). The drug can directly inhibit the ability of purified yeast topoisomerase II to relax supercoiled DNA. Because double stranded DNA is broken and rejoined in the process of strand passage, topoisomerase II becomes covalently linked to the 5' terminus of each strand. Several antineoplastic agents, including amsacrine, doxorubicin, and etoposide, act by stabilizing this cleavable complex formed in the process of topoisomerase II mediated DNA strand passage. In vitro, at 10 μM, suramin completely inhibited cleavable complex formation and inhibited amsacrine and etoposide induced cleavable complex formation. Moreover, suramin does appear to enter the nucleus of hamster fibroblast cells and, at 50 μM, can decrease the number of protein linked DNA strand breaks produced by amsacrine. These data suggest that combination therapy with suramin and a topoisomerase II inhibitor would not be successful.

Selected Preclinical Studies

Prostate Cancer. Studies with suramin in prostate cancer lines in vivo demonstrate that the cytotoxic effects are both highly concentration and cell line dependent. For example (112), at <100 μM, suramin stimulated the growth of the MLL subline of the rat Dunning R3327 tumor; while at higher concentrations, cell growth was inhibited. In the PC3, DU145, and AT2 lines; and in prostatic tumor explants, no effect on growth was found at low suramin concentration, while growth inhibition occurred at higher concentration.

Kim et al. (113) have shown that significant inhibition of androgen independent DU145 cells by suramin (100 or 300 μg/ml) required approximately 5 days. Cells appeared to be blocked in S phase. Both this line and the PC3 line contain EGF receptors and secrete growth promoting TGF-α, which is its natural ligand. Binding of TGF-α to its receptor was blocked by suramin at 400 μg/ml, and this correlated with decreases in cell growth that were partially overcome by treatment with exogenous TGF-α. The ability of TGF-α to only partially overcome the effects of suramin indicates that multiple growth inhibition mechanisms may be active. The androgen dependent line LNCaP was somewhat more sensitive to the cytostatic effects of suramin (114), perhaps because of increased cellular retention of the drug relative to the DU145 line (115). In the LNCaP cells, growth was arrested in the G2-M phase of the cell cycle (116). In androgen dependent DDT-1 cells, suramin (<100 μM) actually stimulated growth in the presence of testosterone but was inhibitory at higher concentrations. In this cell line, the growth stimulatory effects of PDGF and bFGF were essentially entirely ablated by suramin at 100 μg/ml.

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Peehl et al. (117) used an in vitro explant system for culturing prostate cells taken from radical prostatectomies in patients with normal, hyperplastic, or cancerous prostates. At concentrations ranging from 0 to 50 μg/ml, suramin inhibited growth of the cells taken from the hyperplastic and cancerous prostates but not from the normal prostates. The antiproliferative effect was transient and disappeared after 24 h, even at a suramin concentration of 500 μg/ml. Additional experiments designed to evaluate the effects of suramin on normal prostate tissue were designed by Morton et al. (118). They castrated normal male Copenhagen rats and after 10 days treated them with testosterone and suramin or protamine. No inhibition of prostate regeneration occurred with either. However, both inhibited the growth of s.c. implanted AT-2 prostate cells.

Suramin may also affect circulating levels of gonadotropins (118, 119). In rats receiving the drug, plasma testosterone fell to castrate levels. This was not associated with a drop in luteinizing hormone, but FSH did fall, suggesting inhibition of gonadotropin release at the level of the pituitary (118, 120). Furthermore, suramin could also inhibit the binding of FSH to its cell surface receptor at a concentration (about 10 μM) that was similar to that observed for suramin mediated inhibition of FSH-stimulated steroidogenesis in Sertoli cells. Other direct effects on the testes included suppression of luteinizing hormone/human chorionic gonadotropin stimulated steroidogenesis.

**Breast Cancer.** Eisenberger and Fontana (121) have recently discussed literature data that indicated that a variety of growth factors, including TGF-α, IGF-I, and IGF-II, may be potent mitogens for breast carcinoma cells and may act in an autocrine or paracrine factor. Cullen et al. (122), demonstrated that PDGF, secreted by breast tumor cells that do not bear the PDGF receptor, may bind tostromal cells and increase their production of IGF-I, which, as mentioned above, is mitogenic for the breast tumor cells. In estrogen receptor positive MCF-7 cells, suramin (>100 μM) could inhibit growth stimulated by IGF-I, as well as by EGF, 17β-estradiol, and cathepsin D (123). The latter, a lysosomal enzyme, may help promote breast cancer invasion and metastasis. At lower suramin concentrations, suramin increased the mitogenic activity of EGF and cathepsin D. This biphasic response to suramin has also been observed in several other breast cancer lines (124). Estrogen receptor negative cell lines (MDA MB 231, SK-BR-3), were particularly sensitive to the cytostatic effects of suramin (50% inhibitory dose, MDAMB231, 7 μM; MCF7, 50 μM). Clinical trials of suramin in single treatment failure metastatic breast cancer are to begin soon at the University of Maryland.

**Colon Cancer.** An interesting example of the ability of suramin to regulate cellular growth and differentiation is found in the work of Fantini et al. (82, 125-129). These authors have investigated the effects of suramin on the growth and differentiation of the HT29-D4 colonic adenocarcinoma line. These cells undergo differentiation in galactose containing, glucose free media. In the presence of 100 μg/ml suramin, the cells became organized into a polarized monolayer of columnar cells, became antisecretase antibody reactive, and expressed alkaline phosphatase and isomaltase, which are normal brush border hydrolases. Differentiation may have occurred because of the ability of suramin to block the binding of IGF-1 (IC50 25 μg/ml), a postulated autocrine growth factor for this cell line, to its receptor (128). A suramin analogue, NF036 (130), was able to induce HT29-D4 differentiation but did not affect lysosomal morphology (127). This result is highly significant because NF036 contains only a single naphthalene trisulfonate moiety and is much less likely to bind to growth factors with high avidity. Structure-activity studies of this type will be of great help in defining relevant targets for suramin and its analogues.

Levels of secreted tumor markers may also be affected by suramin. Carcinoembryonic antigen, which is normally released from HT29-D4 cells during differentiation, was not released in the presence of suramin (130). Carcinoembryonic antigen appears to be anchored to the cell membrane by a glycane phosphatidylinositol. Analogous observations have been made (131, 132) on the ability of suramin, in the rat C6 glioma line, to prevent the labilization, from the cell surface, of an isoform of the glycane phosphatidylinositol anchored N-CAM.

**Inhibition of the Metastatic Phenotype.** Because of the ability of suramin to inhibit the activity of lysosomal GAG catabolic enzymes, it is reasonable to expect that the drug could inhibit extracellular heparanases as well. Nakajima, et al. (133) showed that melanoma heparanase (an endo-β-D-glucuronidase) could be noncompetitively inhibited by suramin. Suramin also inhibited the degradation of subendothelial basement membrane by B16 melanoma cells, and inhibited invasion. However, no effects on B16 cell growth were observed, suggesting a decoupling between an increase in cell number and metastatic potential. Other sulfated glycoconjugate-binding molecules in the metastatic process include laminin and thrombospondin. These proteins are found in the extracellular matrix and appear to mediate cell attachment, spreading, and migration (134). Suramin, at physiologically attainable plasma concentrations, could inhibit the binding of thrombospondin to sulfatide. The binding of laminin was also inhibited, but at a higher concentration. Melanoma cell adhesion and chemotactic migration in response to thrombospondin and laminin were also suppressed. Prostate cancer cell (LNCaP) motility, an important property of metastasizing cells, is suppressed by suramin (112) and may be restored by addition of bFGF. The use of suramin as an antimetastatic agent in clinical trials has yet to be explored but, on the basis of the preclinical data, may be a most promising field of endeavor.

**Conclusions**

There can be little doubt of the ability of suramin to affect cellular biology and physiology. However, at the present time, it is probably not prudent to claim that the drug exerts its antiproliferative properties by any single mechanism. The specific mechanism of cellular growth inhibition (e.g., blockade of growth factor-receptor binding) is probably highly tissue type dependent. By extension, different mechanisms of action may be relevant in different cells, although it is difficult to predict which on an a priori basis. Furthermore, critical determinants of the mechanism, such as rates of drug intracellular internalization and subcellular compartmentalization, are currently poorly understood. Studies designed to examine the cellular pharmacology of suramin will help in the determination of which of the many intracellular targets of suramin are relevant to its antineoplastic effects. Whether the drug itself will eventually become part of the therapeutic armamentarium is also not clear, although the initial trials are encouraging. Many difficulties, including the optimum dosing schedule, must be worked out. Finally, the imagination of synthetic organic chemists must be directed toward improving the activity of this agent. Suramin analogues will also be useful in clarifying which targets are truly relevant ones. We anticipate great progress in this direction in the near future.

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**References**


Suramin.


Suramin: A Novel Antineoplastic Agent with Multiple Potential Mechanisms of Action

C. A. Stein


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