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Mechanisms of growth stimulation by suramin in non-small-cell lung cancer cell lines

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Abstract *Purpose*: Suramin, a polysulfonated naphthylurea, has been shown to be effective in the treatment of several cancers. We have reported that suramin, at dose concentrations higher than 140 μ*M*, exerts growth-stimulatory effects in several non-small-cell lung cancer (NSCLC) cell lines. The purpose of this study was to examine the mechanisms by which suramin exerts this growth-stimulatory effect in NSCLC cells. *Methods*: NCI-H596 cells were treated with agarose-immobilized suramin, directly or by addition on cell culture inserts, after which growth was determined by [^{3}H]thymidine incorporation. PPADS, a specific purinergic receptor antagonist, was used to determine whether suramin acts via purinergic receptors. The effect of suramin on epidermal growth factor receptor (EGFR) was determined by analyzing receptor phosphorylation and dimerization. XAMR 0721, a suramin analogue containing only one of the two polysulfonated arms, was also analyzed for its effects on growth and EGFR activation. *Results*: Agarose-immobilized suramin stimulated NCI-H596 cell growth, but only when added directly to the cells. When the suramin-conjugated beads were added to the cells on cell culture inserts, which preclude an interaction with the cell surface but allow interaction with the culture medium, there was no effect on proliferation. PPADS had no effect on the growth stimulation by suramin; however suramin treatment resulted in rapid phosphorylation and dimerization of EGFR. Treatment with XAMR 0721 did not affect growth or tyrosine phosphorylation and dimerization of EGFR. *Conclusions*: Suramin need not enter NCI-H596 cells to exert its growth-stimulatory effect, nor is this effect mediated by an interaction with soluble growth factors. Rather, it appears that suramin acts via an interaction with EGFR, but not with purinergic receptors.

Key words Suramin · Growth factor receptors · Non-small-cell lung cancer · Growth stimulation · Agarose immobilization

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Introduction

Suramin is a hexasulfonated, polyaromatic compound [31, 32] that has antitumor activity in many types of cancer cells in culture and in xenografts [2, 9, 16, 31–33]. Clinically, suramin has proven effective when used as a chemotherapeutic agent for the treatment of metastatic prostate cancer refractory to conventional hormonal manipulation and advanced nodular lymphomas requiring systemic therapy [2, 8, 31, 32, 34]. Part of the antitumor effect of suramin may involve the induction of differentiation, as has been shown in human colon adenocarcinoma and neuroblastoma cell lines [18, 27]. Our recent data have demonstrated that suramin induces apoptosis in non-small-cell lung cancer (NSCLC) cell lines [22]. The molecular basis of the antitumor effects of suramin is currently unknown, although multiple potential mechanisms of action have been proposed based on the wide variety of extracellular and intracellular biological effects of suramin. Suramin can function as a competitor of glycosaminoglycan binding [19], and as a P_{2Y} ATP-purinoreceptor antagonist [6]. It can interrupt internal autocrine growth factor loops [10, 27, 29, 33], inhibit the phosphorylating activity of PKC [18, 22], and

downregulate both diacylglycerol kinase and phosphoinositol kinase activities [21]. In the nucleus suramin can inhibit DNA polymerase α [20] and topoisomerase II [3, 15].

Of these properties, the ability of suramin to uncouple growth factors from their receptors has been demonstrated to be partially responsible for its cytostatic effects in many cell types. The interaction of suramin with growth factors is thought to be due to its substantial negative charge [23]. However, the cytostatic properties of suramin are not uniform. We have shown that, at concentrations higher than 200 μg/ml, suramin stimulates growth in some NSCLC cell lines [22]. Furthermore, it has been reported that at concentrations below 50 μg/ml, suramin induces growth of several other cell types, including breast cancer cells [13], several other NSCLC cell lines [5, 25, 28], esophageal and epidermoid carcinoma cell lines [14, 30], as well as both PC12 cells and dorsal root ganglion neurons [17]. Study of the mechanisms of such potentiation has suggested that suramin may activate growth factor receptors, such as epidermal growth factor receptor (EGFR) and nerve growth factor receptor (NGFR) [5, 17, 30] by inducing tyrosine phosphorylation. However, the mechanisms by which suramin interacts with the receptors, thereby stimulating their autophosphorylation, remain unknown.

It is necessary to completely understand the mechanisms by which suramin stimulates growth to avoid any unwanted growth-stimulatory effects when using suramin for antineoplastic treatment. Since suramin has been demonstrated to localize to different cellular compartments [1] and to interact with extracellular, cytoplasmic and nuclear proteins [3, 6, 10, 19–21, 29], its growth-stimulatory effects can be potentially mediated by any of these proteins. In this study, we utilized a novel approach that allowed us to discriminate between the extracellular and intracellular effects of suramin. Using this approach, we demonstrated that the induction of NSCLC cell proliferation by suramin does not depend upon entry of suramin into the cells. Based on the results of our experiments, we suggest that the symmetric structure of the suramin molecule is responsible for dimerization and autophosphorylation of EGFR and for the growth-stimulatory effects of suramin in these cells.

Material and methods

Reagents

RPMI-1640 culture medium, fetal bovine serum (FBS), penicillin/streptomycin and fungizone were obtained from Sigma (St. Louis, MO). Suramin was a generous gift from the National Cancer Institute. Ab-1 (clone 528) and Ab-5 (clone R.1) anti-EGFR antibodies recognizing epitopes within the cell surface domain of human EGFR, XAMR 0721 [8-(3,5-dinitrophenylene carbonyl-imino)-1,3,5-naphthalenetrisulfonic acid, trisodium] and PPADS (pyridoxalphosphate-6-azophenyl-2′4′-disulfonic acid, tetrasodium) were purchased from Calbiochem (San Diego, CA), and disuccinimidyl suberate (DSS) was purchased from Pierce (Rockford, IL). [^{3}H]Thymidine (74.0 Gbq/mmol, 37.0 Mbq/ml) was obtained from NEN-DuPont (Boston, MA), and [^{3}H]suramin (20–50 Ci/mmol) was purchased from Moraveck Biochemicals (Brea, CA). Epidermal growth factor (EGF) was obtained from Beckton Dickinson Co (Franklin Lakes, NJ) and genistein was purchased from Gibco BRL Co (Grand Island, NY). Insulin-like growth factor (IGF) II and platelet-derived growth factor BB (PDGF BB, Calbiochem) were iodinated by the chloramine T method [4] to a specific activity of 150 μCi/μg.

Suramin immobilization

Suramin was immobilized on agarose beads using the PharmaLink immobilization kit (Pierce) according to the manufacturer's protocol. The PharmaLink Immobilization Kit uses the Mannich reaction to immobilize a ligand to a chromatographic support which consists of the condensation of formaldehyde with immobilized diaminodipropylamine (a source of primary amine) and another compound containing an active hydrogen, which in our case was suramin. Immobilization using the Mannich reaction results in very stable covalent bonds. The procedure was 10% efficient resulting in a final concentration of 8.8 μg suramin/μl agarose beads, as determined using [^{3}H]suramin as a tracer. Nonspecific binding of suramin to agarose beads was measured by incubation of [^{3}H]suramin with agarose beads after pretreatment with PharmaLink reagents, and was found not to exceed 0.2%.

Cell culture

Cells of the adenosquamous NCI-H596 NSCLC cell line were grown in RPMI-1640 medium supplemented with 10% FBS at 37 °C in an atmosphere containing 5% CO_2. All experiments were performed on heavily confluent cultures (7 days after reaching confluence) to approximate the cellular environment of the tumor. Suramin and XAMR 0721 were dissolved in RPMI medium without FBS at concentrations of 0–400 μg/ml, and 0–180 μg/ml, respectively.

Where suramin-conjugated agarose beads were used, the cells were overlayed with the beads and incubated for appropriate times. Control cells were overlayed with suramin-free beads pretreated with PharmaLink reagents (PharmaLink-treated beads). Where cell culture inserts (Costar (Cambridge, MA)) were utilized, suramin-conjugated agarose beads were placed in the inserts, and the inserts were positioned over the wells of 24-well plates (Falcon (Becton Dickinson Labware, Lincoln Park, NY)) with cultured NCI-H596 cells. Control cells received the inserts with PharmaLink-treated suramin-free beads. The absence of leaking of beads from tissue culture inserts was microscopically monitored.

Measurement of cell growth

Cell growth was determined by [^{3}H]thymidine incorporation into growing cells. Cells were grown in 24-well plates and incubated with 0–400 μg/ml suramin for various times. [^{3}H]Thymidine was added to the cells at a final concentration of 0.4 μCi/ml. After 1 h of incubation, the radioactive medium was aspirated, the cells were washed with phosphate-buffered saline (PBS) and were then precipitated with ice-cold 10% trichloracetic acid (TCA). Cells were then solubilized in 0.25 *M* NaOH and thymidine incorporation was quantified by scintillation spectrometry. All assays were performed in triplicate.

^{125}I-IGF II and ^{125}I-PDGF BB binding to immobilized suramin

^{125}I-IGF II and ^{125}I-PDGF BB binding to immobilized suramin were evaluated as follows: ^{125}I-IGF II or ^{125}I-PDGF BB (150 μCi/μg) was added at 150 000 cpm per reaction to 100 μl suramin-conjugated beads (880 μg suramin) for 30 min at room temperature

(RT) in 50 m*M* Tris-HCl, pH 7.4, and 0.05% Tween-20. Bound and unbound IGF II or PDGF BB were determined by measuring the soluble and bead-associated radioactivity by gamma counting.

EGFR crosslinking

Suramin-treated and control cells were washed with PBS and surface receptors were crosslinked using DSS at a concentration of 20 μg/ml in 50 m*M* Tris, pH 7.4. DSS was originally dissolved in DMSO to a concentration of 10 mg/ml. The crosslinking reaction was allowed to proceed for 30 min at RT. One volume of TE buffer (10 m*M* Tris-HCl, pH 7.4, 1 mM EDTA) was then added for 15 min at RT to stop the reaction. Cells were washed twice with PBS and harvested as described below.

Immunoprecipitation and Western blot

NCI-H596 cells were washed twice in ice-cold PBS and lysed by the addition of immunoprecipitation buffer (50 m*M* Tris-HCl, pH 7.4, 150 m*M* NaCl, 1% Triton X-100, 1.5 m*M* EDTA, 0.1 m*M* Na_3VO_4, 1 m*M* phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin). EGFR was immunoprecipitated for 1 h at 4 °C using Ab-1-specific monoclonal antibody immobilized on Protein A/Sepharose beads (Pharmacia (Piscataway, NY)). Immunoprecipitated EGFR was eluted with SDS-sample buffer, boiled and subjected to gradient (2–15%) SDS-PAGE. Following electrophoresis, proteins were electrotransferred from the gels onto nitrocellulose membranes (Schleicher and Schuell) Keene, NH. Membranes were blocked with 5% dried milk and stained with either the specific monoclonal antibody to EGFR or with rabbit polyclonal antibody to phosphotyrosine (Upstate Biotech (Lake Placid, NY)). The assay was completed using a horseradish peroxidase/LumiGLO based Western blot kit by New England Biolabs (Beverly, MA). The proteins were visualized after exposing the membranes to Hyperfilm ECL (Amersham (Arilington Heights, IL)).

Statistics

All experiments were performed in duplicate, unless otherwise indicated, and mean values are presented ± standard error. Comparisons between the values were performed using a two-tailed Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

We have previously demonstrated that suramin at doses concentrations over 140 μ*M* (200 μg/ml) can induce proliferation of two NSCLC cell lines, NCI-H358 and NCI-H596 [22]. To explore the mechanisms of the growth-stimulatory activity of suramin NCI-H596, we first examined whether internalization of suramin is necessary for its growth-stimulatory activity. Suramin was immobilized on agarose beads using the Pharma-Link immobilization kit. The efficiency of crosslinking was monitored by using ^{3}H-labeled suramin, and was demonstrated to be about 10%. The nonspecific adsorption of suramin to the beads did not exceed 0.2%. The functional activity of the agarose-bound suramin was verified by the binding of ^{125}I-IGF II to the beads. Beads which were crosslinked with suramin demonstrated high binding of radiolabeled IGF II, whereas control beads did not bind any substantial amount of IGF II with most IGF II remaining in the soluble

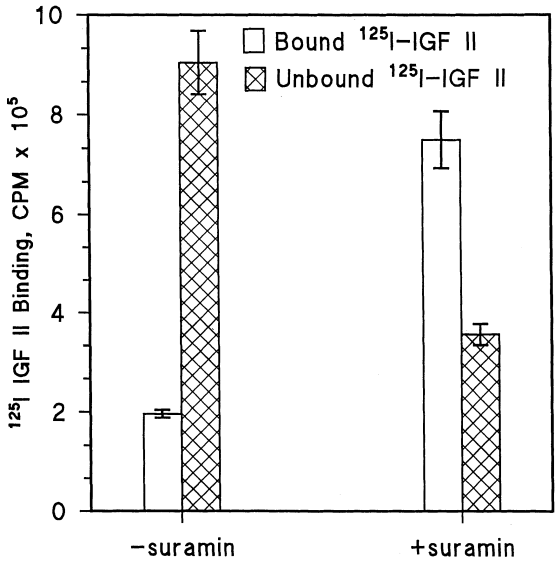

Fig. 1 ^{125}I-IGF II binding to suramin-conjugated beads. Suramin was crosslinked to agarose beads as described in Methods. ^{125}I-IGF II was added to 100 μl suramin-conjugated or control beads for 30 min at room temperature. The amount of bead-associated and soluble ^{125}I-IGF II was determined by gamma counting

fraction (Fig. 1). Analogous results were obtained using iodinated PDGF BB in the binding assay (data not shown). Next NCI-H596 cells were incubated with suramin-conjugated beads for 24 h, after which they were assayed for proliferation. Suramin-conjugated

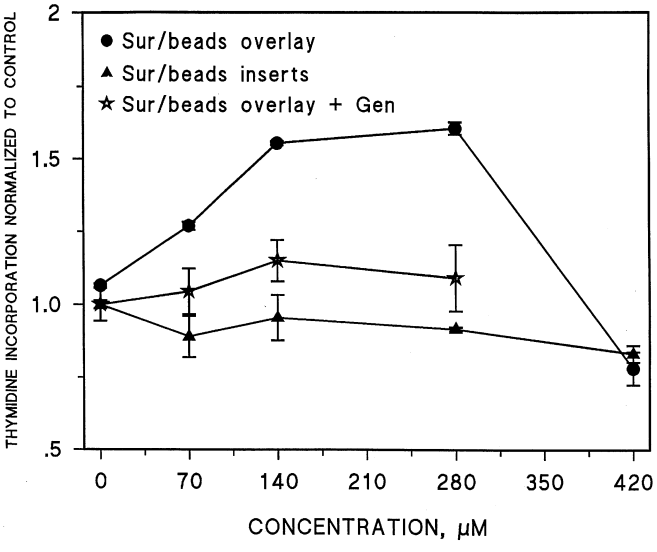

Fig. 2 Thymidine incorporation assay in NCI-H596 cells treated with immobilized suramin. NCI-H596 cells were incubated with the indicated concentrations of suramin immobilized on agarose beads with or without 50 μg/ml genistein (*Gen*). Cells were allowed to contact suramin-conjugated or control beads by direct cell overlay (*Sur/beads overlay*). Alternatively, suramin-conjugated or control beads were spatially separated from the cells by placing the beads on cell culture inserts as described in Methods (*Sur/beads inserts*). Incubation continued for 24 h after which beads were removed and [^{3}H]thymidine incorporation was assayed as above

beads stimulated cell growth in a dose-dependent manner as compared with control PharmaLink-treated beads without suramin (Fig. 2) or with beads bearing nonspecifically adsorbed suramin (data not shown). These results indicate that suramin does not require internalization to exert its growth-stimulatory activity in NSCLC.

Since suramin is characterized by the ability to bind both growth factors and their receptors, as well as P_{2Y}-purinergic receptors, the next experiment tested which of these interactions mediate the effects of suramin. Suramin-conjugated and control agarose beads were placed in the cell culture inserts, and the inserts were positioned over the wells with cultured NCI-H596 cells. The inserts preclude interaction of suramin with the cells but allow interaction with the culture medium. In this way, suramin cannot interact with cellular receptors but can bind growth factors in the medium. Cells were incubated for 24 h, after which thymidine incorporation was determined. Spatial separation of suramin from cultured cells resulted in the loss of its growth-stimulatory activity (Fig. 2). We, therefore, concluded that interaction of suramin with cell surface proteins rather than with soluble growth factors, is necessary for it to stimulate growth.

To examine whether interaction of suramin with P_{2Y}-purinergic receptors could be responsible for its growth-stimulatory effects, we incubated the NCI-H596 cells with increasing concentrations (10–100 μM) of the specific P_{2Y}-purinergic receptor antagonist, PPADS, prior to treatment with suramin. PPADS was not able to inhibit the growth-stimulatory effects of suramin in NCI-H596 cells (Fig. 3), indicating that growth stimulation of NCI-H596 cells by suramin is not mediated by P_{2Y} receptors.

We next examined the possibility that the interaction of suramin with growth factor receptors may mediate its growth-stimulatory effects in NCI-H596 cells. Since many known growth factor receptors contain tyrosine kinase activity, we investigated the growth-stimulatory effect of suramin in the presence of the tyrosine kinase inhibitor, genistein. Exposure to genistein completely abrogated the growth-stimulatory effect of both soluble and immobilized suramin (Figs. 2 and 3) paralleled by an inhibition of EGF-induced EGFR autophosphorylation (Fig. 4A) implicating EGFR tyrosine kinase in the suramin-mediated growth-stimulatory pathway. Pretreatment of NCI-H596 cells with anti-EGFR Ab-5 neutralizing antibody partially abrogated the growth-stimulatory effect of suramin (Fig. 3). These results suggest that the suramin-interactive cell surface proteins are growth factor receptors.

To examine the mechanisms of the effects of suramin on EGFR, we immunoprecipitated NCI-H596 cell extracts with anti-EGFR antibody followed by staining with monoclonal antiphoshotyrosine antibody. We discovered that suramin induces autophoshorylation of EGFR after only 10 min of incubation (Fig. 4A). We analyzed the time-dependent increase in EGFR autophosphorylation using scanning densitometry by normalizing the intensity of each band against the amount of EGFR protein, followed by normalizing of the data for each time-point against the control (0 min) time-point. The resulting increase relative to control in suramin-induced EGFR aurophosphorylation was 2.2, 2.4, 2.2 and 0.8 for 10, 30, 60 and 180 min, respectively. When the same extracts were stained with anti-EGFR antibody, a crossreactive band of about 340 kDa corresponding to EGFR dimers was visualized. As with antiphosphotyrosine binding, the maximal effect of suramin on EGFR dimerization took place after 30 min of incubation (Fig. 4B). When analyzed by scanning densitometry, the increase relative to control in suramin-induced EGFR dimerization was 1.6, 1.8, 1.7 and 1.0 for 10, 30, 60 and 180 min, respectively.

We next utilized XAMR 0721, an analogue of suramin containing only one of the two polysulfonated arms. Incubation of NCI-H596 cells with XAMR 0721 at the various concentrations corresponding to the

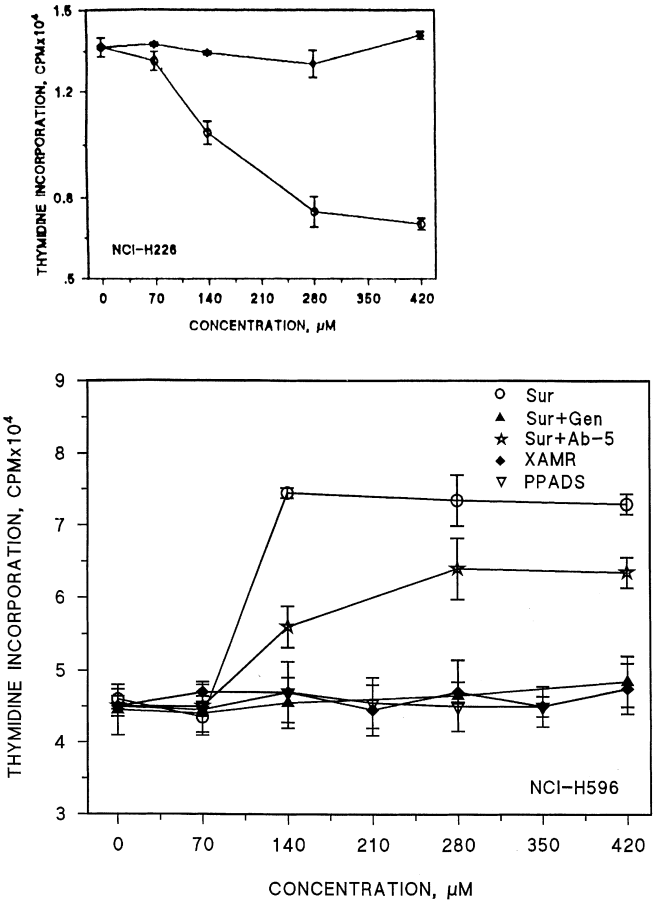

Fig. 3 Thymidine incorporation assay in NSCLC cells. Confluent NCI-H596 cells were preincubated with or without 50 μg/ml of genistein (*Gen*) or 5 μg/ml Ab-5 for 2 h followed by addition of the indicated concentrations of suramin (*Sur*). Incubation continued for an additional 24 h after which [^{3}H]thymidine incorporation was assayed as described in Methods. Alternatively, cells were incubated with the indicated concentrations of XAMR. *Inset*: Proliferation of NCI-H226 cells treated with either suramin or XAMR at the indicated concentrations

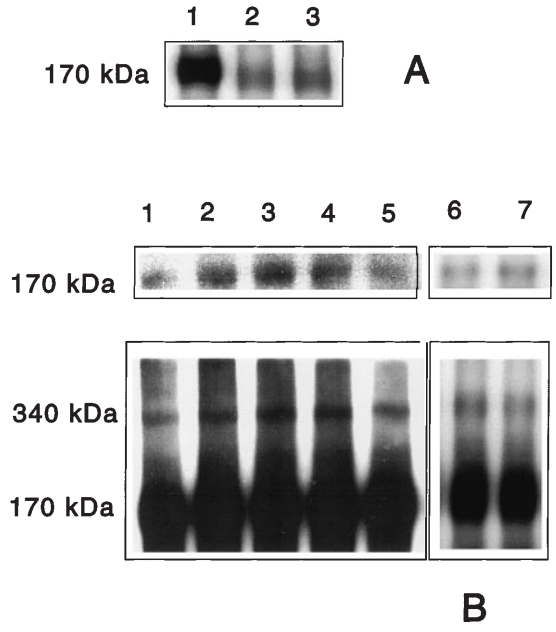

Fig. 4A,B Effect of suramin on EGFR autophosphorylation and dimerization in NCI-H596 cells. **A** NCI-H596 cells were incubated in the presence or absence of 10^{-7} *M* EGF with or without 50 μg/ml of genistein. EGFR was immunoprecipitated, electrophoresed and electroblotted as described in Methods. EGFR autophopshorylation was visualized using rabbit antiphoshotyrosine antibody (*lane 1* EGF-treated cells, *lane 2* control (nontreated) cells, *lane 3* EGF + genistein-treated cells). **B** NCI-H596 cells were incubated with 200 μg/ml suramin in serum-free medium. EGF receptors were crosslinked and EGFR was immunoprecipitated, electrophoresed and electroblotted as described in Methods (*top panel* EGFR autophopshorylation was visualized using rabbit antiphoshotyrosine antibody; *bottom panel* EGFR monomers and dimers were stained with monoclonal anti-EGFR Ab-1 antibody; *lanes 1–5* incubation with suramin for 0, 10, 30, 60 and 180 min; *lanes 6*, *7* incubation with 200 μg/ml of XAMR for 0 and 30 min)

effective suramin concentrations, (0.14–0.56 μ*M*) for 24 h did not affect growth (Fig. 3) or tyrosine phosphorylation and dimerization of EGFR (Fig. 4). Furthermore, XAMR 0721 had no effect on another NSCLC cell line, NCI-H226, the growth of which is inhibited by suramin at these concentrations (Fig. 3, inset).

Discussion

Suramin has attracted the attention of oncologists as the prototype of a new class of anticancer drugs that are able to disrupt autocrine and/or paracrine growth factor stimulatory pathways, thereby slowing cancer growth. Although suramin demonstrates a broad range of effects, the main hypothesis for its chemotherapeutic effect is based on experimental data indicating that suramin directly interferes with receptor/growth factor binding [10, 27, 31–33]. Consequently, the antitumor activity of suramin may result from an inhibition of growth factor-mediated cell proliferation. In agreement with this hypothesis, it has been reported that suramin has an antiproliferative activity against cancer cells expressing EGFR [5, 30]. However, recent studies in several laboratories, including our own, have demonstrated that suramin can induce proliferation of several epithelial cancer cell lines, among them cell lines with high expression of EGFR [13, 14, 25, 28]. The mechanisms of such growth-stimulatory effects of suramin are not completely understood. Although suramin is currently in use as an experimental drug for the treatment of patients with epithelial cell tumors, the apparent lack of success during clinical trials of suramin in NSCLC and advanced breast cancer [24] has raised the question as to whether the growth-stimulatory effects of suramin may be responsible. In the present study we investigated the mechanisms of suramin-induced growth stimulation in NSCLC cell lines.

In the present study, we introduced an experimental approach utilizing immobilization of suramin on agarose beads to discriminate between its extracellular and intracellular effects. The Mannich reaction-based PharmaLink kit which we used allows a wide variety of compounds containing an active hydrogen to be covalently immobilized to a chromatographic support. Suramin contains several active hydrogens that can be crosslinked in the Mannich reaction. We obtained about 10% efficiency of suramin-agarose crosslinking using the PharmaLink kit. The low nonspecific adsorption of suramin to control agarose beads provided the proof that PharmaLink-treated suramin is truly covalently bound to the agarose and not just adsorbed to it. In order to utilize suramin-agarose immobilized with the PharmaLink kit in our experiments, we first had to confirm that the agarose-immobilized suramin remained structurally and functionally unimpaired. Since the Mannich reaction did not involve oxidation, the immobilized suramin did not undergo oxidative decomposition. In addition, since this reaction utilized only an existing active hydrogen on the suramin molecule, no derivatization of suramin was required. Moreover, the functional activity of agarose-bound suramin was confirmed by its binding of two growth factors, IGF II and PDGF BB. Finally, the growth effect of agarose-immobilized suramin in NSCLC cells was similar to the effect of soluble suramin.

We have previously demonstrated that in cell lines from NSCLC with glandular features, suramin significantly stimulates growth at concentrations of 200–600 μg/ml, while having a slight inhibitory effect when applied at concentrations below 50 μg/ml (our unpublished observation, [11). This mode of action of suramin in the two studied NSCLC cell lines is different from its growth-stimulatory activity in breast and esophageal cancers where suramin stimulates growth at lower concentrations and inhibits at higher concentrations [5, 14, 25, 28, 30]. It is unlikely that this difference in suramin activity is the result of differences in EGFR expression, since no such correlation was discovered in experiments using 25 cell lines derived from nine different human cancers [26]. Suramin has been reported to interact with multiple extra- and intracellular proteins [3, 6, 10, 15, 18–22, 27, 29, 33]. In our study, we introduced a novel

experimental approach, whereby immobilized, agarose-bound suramin was used to discriminate between its extracellular and intracellular activities. This approach allowed us to demonstrate, for the first time, that the growth-stimulatory effect of suramin requires only that it interact with the cell surface, and not with intracellular molecules.

Our results indicate that at least part of suramin's growth-stimulatory effect may occur via phosphorylation of EGFR. This agrees with previous reports of the ability of suramin to enhance cell surface signaling via EGFR phosphorylation which coincides with its growth-stimulatory effects [5, 30]. We have also demonstrated that in circumstances where suramin inhibits growth in other NSCLC cell lines, it also inhibits autophosphorylation of EGFR (our unpublished observations) emphasizing the correlation between the growth the effects of suramin and the degree of EGFR autophosphorylation. In the present study, we expanded the evidence to include the finding that suramin-induced phosphorylation of EGFR coincides with the increased dimerization of EGFR. Therefore, in these cell lines, suramin at concentrations above 140 μ*M* (200 μg/ml) acts as an EGFR agonist. Since incubation with anti-EGFR neutralizing antibody did not completely reverse the growth-stimulatory effect of suramin, and considering that suramin can potentially interact with receptors of several growth factors [5, 7, 12, 14, 17, 30], it is likely that suramin also interacts with growth factor receptors other than EGFR in NSCLC cells.

It appears that it is the symmetrical nature of the suramin molecule that allows the dimerization and activation of EGFR upon binding. Of several suramin analogues examined for their ability to inhibit growth in various human cells, only the symmetric compounds exhibited antiproliferative activity [11]. Similarly, XAMR 0721, a suramin analogue that, although it is negatively charged, constitutes only one of the polysulfonated arms of the suramin molecule, has no effect on the growth of NCI-H226, NCI-H596, or NCI-H358 cell lines (unpublished observation). We, therefore, believe that it is the symmetric nature of suramin that is responsible for receptor binding and activation in NSCLC cells. However, until the parameters determining whether this results in growth stimulation or inhibition are fully understood, suramin should be used with caution in the treatment of NSCLC patients.

In conclusion, we introduced an experimental approach utilizing immobilized agarose-bound suramin to discriminate between its extracellular and intracellular effects. Such an approach could be potentially useful in sorting out the mechanisms of other anticancer activities of suramin. Moreover, immobilization of suramin in clinical applications may help to avoid the severe side effects following systemic infusion and aid in the specific targeting of the drug. Using this experimental approach, we demonstrated for the first time that the induction of NSCLC cellular proliferation by suramin does not depend upon entry of suramin into the cells. Our experiments also showed that suramin's growth-stimulatory effects not only correlate with EGFR autophosphorylation, but also require receptor dimerization. We suggest that the symmetric structure of the suramin molecule is responsible for dimerization and autophosphorylation of EGFR and for the growth-stimulatory effects of suramin in NSCLC cells.

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