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Specialist Interest Articles

Antiproliferative Effects of Suramin on Androgen Responsive Tumour Cells

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The effect of the polyanionic drug suramin on two androgen responsive tumour cell lines was studied. Human prostate tumour (LNCaP) cell growth is stimulated two- to three-fold by the synthetic androgen R1881 (0.1 nM) or EGF (1 ng/ml). Suramin (0.01–1.0 mM) inhibited the growth of LNCaP cells in a dose dependent way, both in the presence and absence of androgen or EGF. Growth was arrested in the G0/G1 phase of the cell cycle, but was resumed after removal of suramin. DDT-1 hamster ductus deferens tumour cells are stimulated by PDGF (25 ng/ml), b-FGF (10 ng/ml) and testosterone (10 nM). Suramin inhibited PDGF and b-FGF stimulated cell growth. However in the presence of testosterone, suramin showed a biphasic effect: stimulatory at low dose (0.01 mM) and inhibitory above 0.01 mM. Suramin decreased the apparent affinity of EGF binding sites on LNCaP cells with a two- to eight-fold increase in K_d at 0.1 and 1.0 mM suramin, respectively. In conclusion: suramin counteracts the growth stimulatory effects of both androgens and growth factors on androgen sensitive tumour cells. The effects are reversible after withdrawal of suramin.
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INTRODUCTION

STEROID HORMONES and growth factors are involved in the complex regulation of cell proliferation of hormone sensitive tumours. For example, in human breast cancer cells, oestrogens exert their action through coordinated control of production and secretion of a collection of growth factors [1]. These growth factors act in an autocrine or paracrine way by binding to their cell surface receptors. In addition, steroid hormones may control cell proliferation by modulating the affinity or level of expression of growth factor receptors [2, 3].

Modalities in treatment of cancer patients directed to interference with growth factors or growth factor receptors are scarce. Recently, preliminary data on the application of growth factor antagonists have been reported [4, 5]. One of the compounds, suramin, may be of interest for clinical practice. Suramin is a polysulphonated naphthylurea (M_r: 1429) that has been used since 1920 for the treatment of African trypanosomiasis and onchocerciasis [6–8]. Suramin has been shown to act as a polypeptide growth factor antagonist for platelet derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF) and transforming growth factor beta (TGF-β) [9]. More detailed studies have revealed that suramin acts through inhibition of binding of PDGF to its receptor and by dissociation of bound PDGF from its cell surface receptor [10].

The purpose of the present investigation was to study the effects of suramin on androgen responsive tumour cell lines from epithelial (LNCaP: derived from a metastatic lesion of a human prostatic tumour) and mesenchymal (DDT-1: derived from a leiomyosarcoma of the ductus deferens of a Syrian hamster) origin. In DDT-1 and LNCaP cells androgens, growth factors and their receptors play an important role in the regulation of cell proliferation [11–14].

This article shows that suramin can counteract the growth stimulatory effects of both androgens and growth factors on DDT-1 and LNCaP cells.

MATERIALS AND METHODS

Steroid hormones and growth factors

8,8'- (Carbonylbis(imino-3,1-phenylenecarbonyl- imino(4-methyl-3, 1-phenylene)carbonyl-imino))bis -1,3,5-naphtalenetrisulfonic acid hexasodium salt, Suramin, a product from Bayer AG, was obtained from Mobay Chemical Corporation (New York, NY).

The synthetic androgen R1881 (17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one) was purchased from New England Nuclear (Boston, MA). EGF from mouse submaxillary gland and testosterone were obtained from Sigma Chemical Company (St Louis, MO, U.S.A.). PDGF was receptor grade (90%) pure from Seragen, Boston, MA, U.S.A. b-FGF was obtained from Collaborative Research Inc., Bedford, MA, U.S.A. Mouse [^{125}I]EGF (specific activity: 140 Ci/g, Amersham, U.K.) was used after removal of free iodine by Sephadex G25 gel filtration.

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Cell culture

LNCaP cells, a gift from Dr J. Horoszewicz (Buffalo, NY) were cultured as described previously [13]. LNCaP cells between passage 65 and 70 were used for our experiments.

DDT-1 cells were routinely cultured as described by Syms *et al.* [11]. Medium was changed every 3 days.

Growth studies

LNCaP. Cells were plated in 24 multi-well dishes (Falcon, Oxnard, CA) at a density of 2×10^4 cells/cm^2 in RPMI 1640 medium supplemented with 5% (v/v) charcoal-treated foetal calf serum. The cells were allowed to attach and initiate growth for 3 days. The medium was changed and cells were grown in the presence or absence of different concentrations of suramin (0.01–1 mM) with or without 0.1 nM R1881 or 1 ng EGF/ml (one medium change after 3 days). At day 6 cells were used to measure DNA content according to Hinegardner [15].

To study whether the effect of suramin on LNCaP cell proliferation is reversible, cells were plated in T25 flasks (Nunc, Roskilde, Denmark) in RPMI 1640 medium containing 5% (v/v) charcoal treated foetal calf serum at a density of 2×10^4 cells/cm^2 and allowed to attach for 3 days. Medium was changed and the cultures received suramin (0.1 mM) with either 1 ng EGF/ml or 0.1 nM R1881. After 6 days some of the cultures were rinsed four times with PBS and dissolved in 1 M NaOH for estimation of DNA content. The remaining cultures were rinsed with PBS and grown in the presence or absence of 1 ng EGF/ml or 0.1 mM R1881 with or without 0.1 mM suramin for another 11 days (medium change every 3 days) and DNA content per culture was measured as described.

DDT-1. DDT-1 cells were plated in DME/HAM-F12 (1:1) supplemented with 0.1% bovine serum albumin (BSA) and insulin (5 μg/ml), transferrin (5 μg/ml) and sodium selenite (5 ng/ml, ITS, Sigma, St. Louis, MO, USA). The cells were allowed to attach for 24 h and the medium was replenished with the same medium without 0.1% BSA. Cell growth was continued for 7 days with various concentrations of suramin (0–1.0 mM) in the presence or absence of 10 nM testosterone or PDGF (25 ng/ml) or b-FGF (1–10 ng/ml). Medium was changed every 48 h. Finally cells were harvested and counted using a Coulter counter.

Flow cytometry

Flow cytometry was performed as described before [16]. Briefly, bromodeoxyuridine, a marker for DNA synthesis (BrdUrd, Serva, Heidelberg, F.R.G.) was added to the LNCaP cells 30 min before harvesting. Cell nuclei were isolated and incubated both with FITC labelled anti-BrdUrd and propidium iodide (a marker for the amount of DNA). Suspended nuclei were analyzed by dual parameter flow cytometry.

Excitation and emission wavelengths of FITC and propidium iodide were 494;517 and 540;625 nm respectively. For each sample at least 10^4 events were analyzed.

EGF binding assay

EGF binding assays were performed as described previously [14]. Duplicate cell cultures were incubated with [^{125}I]EGF plus unlabelled EGF (final EGF concentration/well ranged from 0.1 to 30 nM) in the presence or absence of suramin for 4 h at 4°C. Radioactivity of the pellets was measured and Scatchard analysis of binding data was performed after correction for non-specific binding.

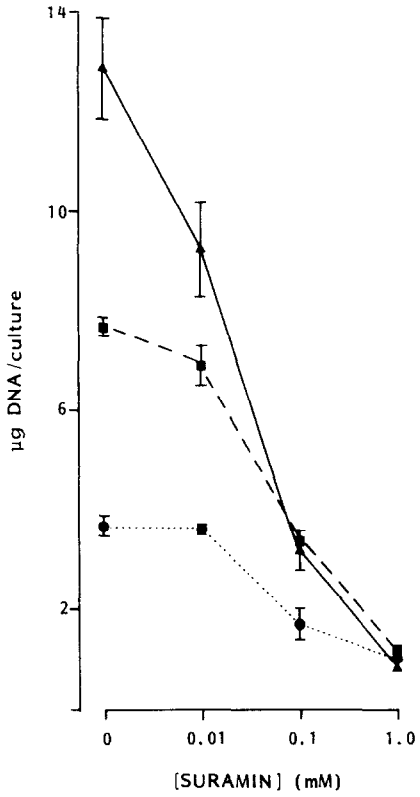

Fig. 1. Effects of suramin and growth factors on growth of LNCaP cells. LNCaP cells were cultured for 6 days in the presence of various concentrations of suramin (0–1 mM) in RPMI-1640 supplemented with 5% (v/v) charcoal treated foetal calf serum only (●—●) or with added 1 ng EGF/ml (■—■) or 0.1 nM R1881 (▲—▲). Data represent mean DNA content of triplicate cultures with standard deviation. At the start of the culture periods, all cultures contained 1.0± 0.1 μg DNA.

RESULTS

Short term effects of suramin on growth of LNCaP and DDT-1 cells

LNCaP cells were cultured for 6 days with various concentrations of suramin, either in the absence or presence of 0.1 nM R1881 or 1 ng EGF/ml (Fig. 1). R1881 and EGF stimulated LNCaP cell proliferation three- and two-fold respectively (growth stimulation expressed as increase of DNA content per culture vs. control cultures). Suramin inhibited the growth stimulatory effects of EGF and R1881 in a dose dependent manner (Fig. 1). Growth of control cultures was also inhibited by suramin. Already at a concentration of 0.01 mM, suramin partially inhibited the growth stimulatory effect of R1881 while the growth stimulatory effect of EGF was only slightly affected. At 0.1 mM, suramin inhibited the growth simulatory effect of both EGF and R1881 (Fig. 1). Suramin completely blocked LNCaP cell proliferation at a concentration of 1 mM even in the presence of EGF or R1881 (Fig. 1).

The inhibitory effect of 0.1 mM suramin could not be overcome by increasing doses of EGF or R1881. At 1, 10 or 100 ng EGF/ml in the presence of 0.1 mM suramin (triplicate cultures per EGF concentration), DNA content in 6 day cultures was not significantly different. Also, DNA content per culture was not significantly different when, in the presence of 0.1 mM suramin, increasing concentrations of R1881 (0.01, 0.1, 1.0 or 10 nM) were added to LNCaP cells.

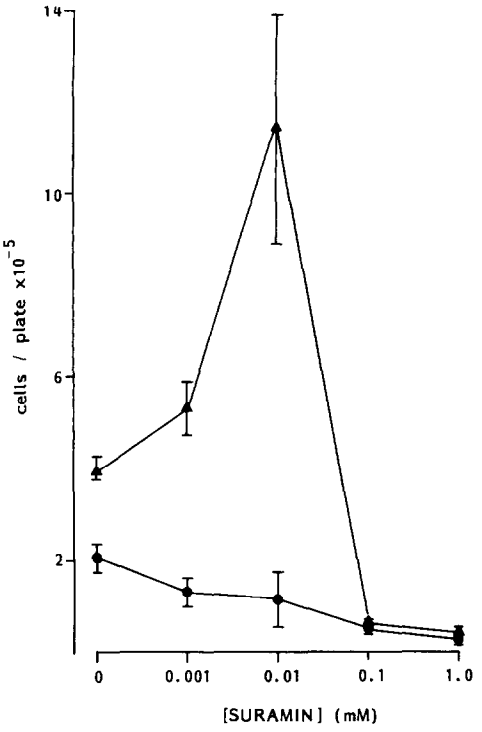

Fig. 2. Effect of suramin on DDT-1 cells cultured in the presence or absence of testosterone. DDT-1 cells were cultured for 7 days in the presence of various concentrations of suramin (0–1 mM) in DMEM/F12 plus ITS (see Materials and Methods) without (●—●) or with 10 nM testosterone (▲—▲). Data represent mean cell number of triplicate cultures with standard deviation. At day zero 10^4 cells were seeded.

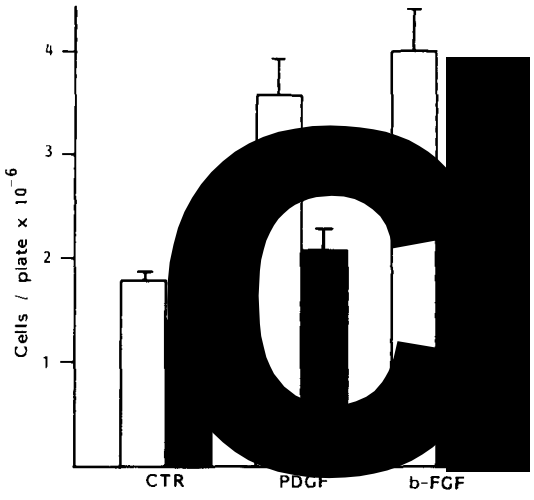

Fig. 3. Effect of suramin on DDT-1 cells cultured in the presence and absence of growth factors. DDT-1 cells were cultured for 7 days in medium as described in Fig. 2, control, or in the presence of PDGF (25 ng/ml) or b-FGF (10 ng/ml) without (open bars) or with (hatched bars) suramin (0.1 mM). Data represent mean cell number of triplicate cultures with standard deviation. At day zero 10^5 cells were seeded.

DDT-1 cells were cultured for 7 days with or without 10 nM testosterone in the presence or absence of suramin. Suramin inhibited the growth of cells cultured in the absence of testosterone in a dose dependent manner (Fig. 2). A two-fold increase in cell number compared to control was observed upon stimulation with 10 nM testosterone. In the presence of testosterone the effect of suramin was biphasic. At low concentrations of suramin (0.001–0.01 mM) DDT-1 cell proliferation was stimulated while at higher concentrations (above 0.1 mM) cell proliferation was inhibited. Suramin completely blocked DDT-1 cell proliferation at a concentration of 1 mM. Addition of PDGF (25 ng/ml) or b-FGF (10 ng/ml) to the culture medium stimulated DDT-1 cell proliferation two-fold. Suramin (0.1 mM) strongly inhibited the growth stimulatory effects of PDGF and b-FGF (Fig. 3), whereas a 10-fold increase in growth factor concentration resulted only in a slight increase (between 10 and 15%) of DDT-1 cell growth (not shown).

Table 1. *Long term and reversible effects of suramin on growth of LNCaP cells*

Culture conditions	μg DNA/culture Day 6	Day 17	
	(a)	(b)	(c)
Suramin	+	+	–
Control	37.4	32.2	47.2
1 ng EGF/ml	36.6	14.1	82.4
0.1 nM R1881	41.6	6.6	74.2

Cells were cultured for 6 days in the presence of 0.1 mM suramin (control) or with 1 ng EGF/ml or 0.1 nM R1881 (column a). Thereafter growth was continued as indicated in the presence (column b) or absence (column c) of suramin. Data represent the mean of duplicate experiments.

Long term and reversible effects of suramin on LNCaP cell growth

The effect of prolonged exposure (over 6 days) of LNCaP cells to 0.1 mM suramin was studied. Table 1 shows that DNA content per culture decreased, when suramin was added. Interestingly, after 17 days of culture, this decrease in DNA content was more pronounced in cell cultures treated with suramin in the presence of growth stimulatory factors (EGF or R1881) when compared to cell cultures treated with suramin alone. Table 1 also shows that withdrawal of suramin from the cell cultures after 6 days allows recovery of growth stimulation by EGF or R1881. Furthermore, after pretreatment with suramin for 6 days, the increase in DNA in the second culture period upon stimulation with 1 ng EGF was approximately equal to stimulation by 0.1 nM R1881. This is in contrast with the observations that androgens are more potent mitogens for LNCaP cells when compared to EGF if cells are cultured for 6 days in the absence of suramin (Fig. 1).

Effects of suramin on cell cycle kinetics of LNCaP cells

The effects on cell cycle kinetics of suramin in the presence and absence of 1 ng EGF/ml or 0.1 nM R1881 were studied. Cells were allowed to attach and initiate growth for 3 days. The percentage of cells in G0/G1 phase and S phase after these 3 days was 74 ± 1 and 17 ± 1 respectively (mean and standard deviation, n = 3). Cells were grown for 24 h in the presence or absence of EGF or R1881 with or without 1.0 mM suramin. After treatment of LNCaP cells with EGF or R1881 the percentage of cells in the S phase increased about two-fold whereas the percentage of cells in the G0/G1 phase decreased compared to control cells (Fig. 4). A decrease in the percentage of cells in S phase was observed after treatment of the cells with 0.1 mM

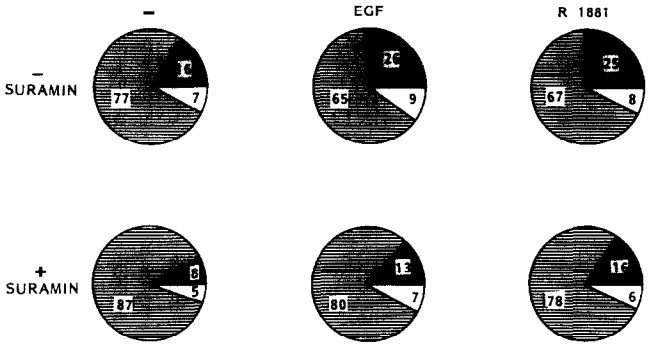

Fig. 4. Dual parameter flow cell cytometric analysis of LNCaP cells cultured for 24 h in the absence (control) or presence of 0.1 nM R1881 or 1 ng EGF/ml with and without 1 mM suramin. Percentages of cells in S phase (black), G1/O phase (hatched) and G2/M phase (dotted) are shown (mean of three experiments) in a pie-shaped diagram. Standard deviations (not shown) were less than 8% of the mean.

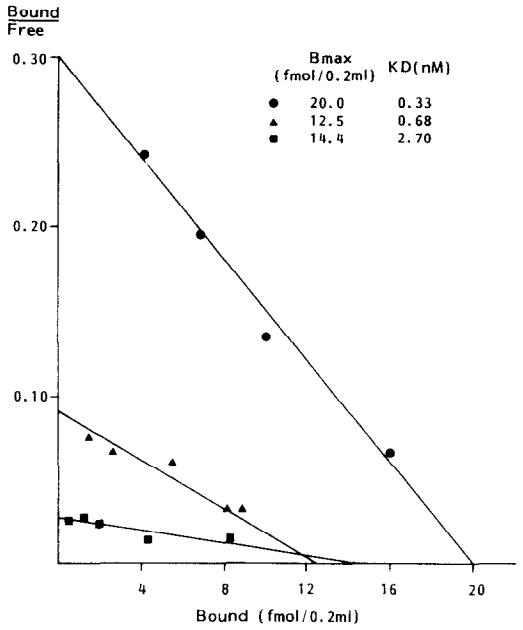

Fig. 5. Scatchard plot analysis of EGF binding to LNCaP cells in the presence or absence of suramin. Binding data were obtained after incubation with [^{125}I]EGF in the absence (●) or presence of 0.1 mM suramin (■) or 1.0 mM suramin (▲) at 4°C for 4 h. The concentration of bound ligand is expressed as fmol/well with 0.2 ml medium. Each well contained 3 × 10^5 LNCaP cells.

suramin either in the presence or absence of EGF or R1881. Upon treatment with suramin the percentage of cells in G0/G1 phase increased while the percentage of cells in G2/M was only slightly or not affected by suramin. When cells were cultured for 48 h a comparable inhibitory effect of suramin on cells in S-phase was observed (not shown).

Effects of suramin on EGF binding to LNCaP cells

The mechanism by which suramin inhibits the growth response of LNCaP cells to EGF was studied in more detail. EGF binding data were obtained by incubation of LNCaP cells with increasing concentrations of labelled and nonlabelled EGF in the absence or presence of suramin. The binding data were well fitted by a one-site binding model resulting in linear Scatchard plots (Fig. 5). Incubation of LNCaP cell cultures with EGF in the absence of suramin revealed a high affinity EGF receptor with a K_d of 0.33 nM. When the EGF binding assays were performed in the presence of 0.1 or 1 mM suramin, the K_d of the EGF receptor increased two- and eight-fold, respectively (Fig. 5). In addition, the number of measurable EGF binding sites was decreased.

DISCUSSION

The purpose of the present investigation was to study the effects of suramin, a polyanionic compound that blocks the interaction of heparin binding (tumour) growth factors like PDGF, b-FGF and TGF-β with their cell surface receptors [9, 10, 17], on androgen responsive tumour cell lines. DDT-1 cells respond to androgens, PDGF or b-FGF with an increase in cell growth. In addition, these cells secrete immunoreactive PDGF [11, 12]. The proliferation of LNCaP cells can be stimulated by both androgens and EGF. Androgens augment this growth response to EGF by increasing the number of EGF receptors expressed at the cell surface [13, 14].

High concentrations of suramin (0.1 and 1 mM) inhibited androgen and growth factor stimulated LNCaP and DDT-1 cell growth. This inhibition is not affected by the concentration of growth factor or steroid. It is therefore not clear through which mechanism suramin acts. Our binding data indicate an apparent decrease in binding affinity of EGF for the EGF receptor of LNCaP cells. The lower amount of binding sites measured in the presence of suramin (0.1–1 mM) is probably due to a lower availability of the growth factor, since it has been shown that suramin binds to EGF in the concentration range used [9]. This by itself may alter the apparent bound drug concentration, or alternatively, a suramin–EGF complex may still bind to the EGF receptor but with altered affinity. We also observed an arrest in G0/1 phase of the cells, probably due to withholding of the progression factor EGF.

Low concentrations of suramin had a growth stimulatory effect on DDT-1 cells. Stimulatory effects of suramin, at low concentration, have been observed also by others [9], indicating that other (indirect) mechanisms may be involved. It has been shown that suramin inhibits the action of TGF-β at relatively low concentrations, however the DDT-1 cell growth is not affected by this factor. DDT-1 cells do not contain mRNA with homology to v-sis, the viral oncogene coding for PDGF like proteins, and also secrete immunoreactive PDGF. Huang and Huang [18] suggested that suramin not only reversed the rapid turnover of the PDGF receptor in sis-transformed cells but also increased the secretion of the sis-gene product (which codes for the b-chain of PDGF). A similar mechanism may hold true for DDT-1 cells. Our *in vitro* studies suggest that suramin may exert its anti-tumour effect on androgen responsive cell lines directly by binding to the tumour growth factors. *In vivo* studies on suramin suggest an action as an anticoagulation factor and inducer of tumour shrinkage [19]. Recently, Myers *et al.* [20] using suramin reported clinical responses in treatment of cancer of the prostate, kidney, adrenal gland and in heavily pretreated non-Hodgkin lymphoma.

In conclusion, suramin may have potential in cancer therapy to inhibit autocrine and paracrine stimulation of androgen responsive tumours, although further studies using *in vivo* models (e.g. Dunning or PC-82 tumour model) are necessary to give better insights or biological effects of suramin.

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Immunoscintigraphy of Hodgkin's Disease: *In Vivo* Use of Radiolabelled Monoclonal Antibodies Derived from Hodgkin Cell Lines

Patrice Carde, Ligia Da Costa, Luc Manil, Michael Pfreundschuh, Jean-Denis Lumbroso, Jean-Claude Saccavini, Bernard Caillou, Marcel Ricard, Frédéric Boudet, Marcel Hayat, Volker Diehl and Claude Parmentier

The Hodgkin associated monoclonal antibody (Mab) HRS-1 reacts with Hodgkin and Reed–Sternberg cells (HR-S) in all HD subtypes. HRS-1 Mab was labelled with radioiodine and injected into 10 patients for immunoscintigraphy (IS). Seven patients were injected with HRS-1 Mab radiolabelled with ^{131}I and three patients were injected with HRS-1 Mab labelled with ^{123}I. A control anti-alpha-fetoprotein (anti-AFP) Mab was radiolabelled with another iodine isotope and was injected simultaneously in five cases. Six out of eight patients with proven HD had a true positive scan (nodal, splenic and bony involvement). Imaging was equivocal or failed in the two other patients. In the last two patients IS imaging was truly negative due to the absence of residual HD in one patient and to an erroneous histological diagnosis of HD in another patient. These results, although preliminary, demonstrate that IS with radioiodine-labelled HRS-1 Mab is feasible and may prove to be informative in the staging of HD.

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