THE EFFECT OF SCHEDULE, PROTEIN BINDING AND GROWTH FACTORS ON THE ACTIVITY OF SURAMIN

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Suramin has been shown to have antiproliferative activity, either by blocking the binding of growth factors to their receptors or by inhibiting critical cellular enzymes. In 6 different cell lines from 5 tumour types (MCF-7, MCF-7/ADR^R, PC3, HT-29, UM-SCC-11B and SW-1573/IR500), we studied the effect of scheduling of suramin, of FCS and of human serum albumin (HSA), of epidermal growth factor (EGF) and of the addition of growth factors in serum-free medium on the activity of suramin. The concentration of suramin which gave 50% growth inhibition (IC₅₀) varied from 45 μ M in SW-1573/IR500 to 153 μ M in PC3 cells grown in medium supplemented with 5% FCS, after 6 days of continuous exposure. Exposure for more than 4 days did not enhance the sensitivity to suramin, except in PC3. At exposure to suramin for 1 day followed by 5 days recovery, high IC₅₀ values (>0.5 mM) were observed in MCF-7 cells. In medium with 1% and 0.5% FCS these values were 3 to 8 and 14 to 26 times lower respectively. Addition of HSA increased the IC₅₀ in PC3 and MCF-7 cells. Suramin binding to protein was dependent on the concentration of protein and of suramin. Excess of EGF in medium with different FCS concentrations did not change the IC₅₀ values of suramin in PC3 and MCF-7 cells. Addition of EGF, fibroblast growth factor or platelet-derived growth factor in PC3 cells cultured in serum-free medium did not increase the IC₅₀ values. Suramin was active against these 6 cell lines at clinically achievable concentrations. This activity varied depending on the cell line, exposure time and suramin concentration. The most significant factor interfering with sensitivity to suramin was the amount of protein present in the culture medium.

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Suramin was synthesized by Bayer (Leverkusen, Germany) at the beginning of the century in a research programme on ureas of the aminonaphthalene-sulfonic type. It has since then been used in the treatment of trypanosomiasis and other parasitic diseases such as onchocerciasis (Hawking, 1978). Suramin is a compound for which a number of different mechanisms of action have been described. These include the inhibition of many different enzyme systems (Eisen and Loveday, 1973).

Suramin is a potent inhibitor of the RNA reverse transcriptase of several oncornaviruses (De Clercq, 1979), and it has *in vitro* activity against human immunodeficiency virus (HIV) (Mitsuya *et al.*, 1984). Suramin was therefore studied in patients with the acquired immune deficiency syndrome (AIDS), but the treatment was accompanied by excessive toxicity and the results were disappointing (Broder *et al.*, 1985).

In one patient with AIDS-related Kaposi's sarcoma and non-Hodgkin's lymphoma there was, however, a complete remission of both tumour types (Cheson *et al.*, 1987). Following these observations, several clinical trials were started in patients with solid tumours, and responses have been reported in prostate, lymphoma and adrenocortical cancers (Stein *et al.*, 1989; LaRocca *et al.*, 1990). The treatment using administration of suramin for prolonged periods of time or at higher dose intensity than used for the treatment of trypanosomiasis revealed several new types of toxicity of suramin, including coagulopathy, polyneuropathy and adrenal failure (LaRocca *et al.*, 1990).

In vitro studies demonstrated that suramin was able to inhibit the growth of a large number of human cell lines, including lymphoid, non-small-cell lung cancer, gynaecologic cancer, breast cancer and prostate cancer cells at concentrations which are clinically achievable (La Rocca *et al.*, 1990).

Suramin has been demonstrated to be highly protein bound (>99%), mainly to albumin ($\pm 80\%$) (Collins *et al.*, 1986; Vansterkenburg *et al.*, 1989). This protein binding is very likely to affect the cytostatic effects of suramin. Because of the long half-life of suramin in serum of treated patients, different scheduling strategies have been used. In addition, the contribution of growth factor concentrations on the *in vitro* and *in vivo* activity of suramin by either increasing or decreasing its anti-tumour activity has not yet been completely elucidated (LaRocca *et al.*, 1990).

For these reasons we determined the growth inhibitory effects of suramin in a variety of cell lines with different histological origins. The effect of scheduling, binding to protein present in the culture medium and to exogenous serum albumin has been studied. In addition the interaction of FCS with the effect of growth factors has been studied.

MATERIAL AND METHODS

Chemicals and reagents

Suramin (Germanin) was purchased from Bayer and stored at 25 mM dissolved in 0.9% NaCl at -20°C . In these conditions the drug was stable for at least 6 months. Appropriate dilutions were prepared just before use. Dulbecco's modified Eagle's medium (DMEM) was purchased from Flow (Irvine, UK); 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) was from Serva (Heidelberg, Germany); FCS and Hanks's balanced salt solution (HBSS) without calcium, magnesium and phenol red were from GIBCO (Grand Island, NY); trichloroacetic acid (TCA), glutamine and gentamicin were from Merck (Darmstadt, Germany); trypsin, (ethylenedinitro)-tetra acetic acid (EDTA), 3-(4,5-dimethylthiazol-2-yl)-, 5-diphenyltetrazolium bromide (MTT), trypan blue, sulforhodamine B (SRB), DMEM with nutrient mixture F-12 (DME/F-12), insulin-transferrin-sodium-selenite media supplement (ITS), trypsin inhibitor, epidermal growth factor (EGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) were all from Sigma (St. Louis, MO); spectrophotometric graded dimethylsulfoxide (DMSO), acetic acid, tris-(hydroxymethyl)-aminomethane (Tris) were from Baker (Deventer, Holland). Human serum albumin (HSA) was from the Central Laboratory of the Blood Transfusion Centre of the Netherlands Red Cross (Amsterdam, The Netherlands). All other chemicals were of standard analytical quality and are commercially available.

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Cell lines

The prostate cancer cell line, PC3, and the colon cancer cell line, HT29, were obtained from the ATCC (Rockville, USA). The squamous-cell larynx cancer cell line UM-SCC-11B was obtained from Dr. T. Carey (Ann Arbor, MI). The breast cancer cell line MCF-7 and its multidrug-resistant variant, MCF-7/ADR^R were obtained from Dr. K.H. Cowan (NCI, Bethesda, MD). The human non-small-cell lung cancer cell line SW-1573/1R500 (originally isolated by Dr. A. Leibovitz, Scott and White Clinic, Temple, TX) was selected and derived as doxorubicin-resistant. Unless otherwise specified, the cells were grown as monolayer cultures in 20 mM HEPES-buffered DMEM supplemented with 5% heat-inactivated FCS and 1 mM L-glutamine, in 80-cm² flasks (Nunc, Roskilde, Denmark) in a 37°C, 5% CO₂, 95% humidified air incubator and were sub-cultured once a week. All cells were free of Mycoplasma infection, as tested by Hoechst staining of a Mycoplasma-sensitive indicator strain of monkey kidney cells (Flow).

Chemosensitivity tests

Cells in the exponential growth phase were harvested with 0.25% trypsin-EDTA, and re-suspended in medium with 50 µg/ml gentamicin. Only suspensions with a viability > 90% (as determined by Trypan-blue exclusion) were used and, after counting with an automatic counter (Sysmex, CC-110, Tokyo, Japan), seeded into 96-well plates (Greiner, Solingen, Germany). The plating densities were PC3, 14,000 cells/well; MCF-7 and SW-1573/1R500, 4,000 cells/well; MCF-7/ADR^R and UM-SCC-11B, 10,000 cells/well and HT29, 5,000 cells/well. Suramin diluted in culture medium was added to the cells 24 hr after seeding.

The SRB assay as developed by Skehan *et al.* (1990) was performed as described by Keepers *et al.* (1991). Briefly, ice-cold TCA (50 µl 50%/well; final concentration 10%) was added to the wells to precipitate the proteins and fix the cells (1 hr at 4°C). After washing, the cells were stained for at least 15 min with 0.4% SRB (dissolved in 1% acetic acid). Unbound stain was removed with 1% acetic acid (4 wash steps) and the protein stain was solubilized with 150 µl 10 mmol/l unbuffered Tris base. The optical density was read at 540 or 510 nm using a microtitre plate reader (Titertek Multiskan MCC/340, Flow) connected to an Olivetti PC M19 microcomputer. Data were collected on floppy disk using TiterSoft EIA software version 2.0A (Flow) and data analysis was done with Symphony 1.2 (Lotus). IC₅₀ values were defined as those concentrations of suramin which produced 50% growth inhibition (Keepers *et al.*, 1991).

We used a modification of the microculture tetrazolium assay (MTA) (Alley *et al.*, 1988) as described by Keepers *et al.* (1991). Briefly, at the end of the tests, medium was aspirated, fresh medium and subsequently 20 µl MTT (final concentration 0.5 mg/ml) were added and the plates were incubated at 37°C, 5% CO₂ for 4 hr. After removal of the supernatant the insoluble formazan crystals were solubilized with 150 µl DMSO containing 0.5% FCS. The plates were shaken intensively and the optical density was measured at a 540 nm filter and the data were analysed as described for the SRB assay.

Determination of protein binding

The determination of protein binding in serum by membrane dialysis was performed as described by Vansterkenburg *et al.* (1989). Briefly, 1 ml culture medium was placed in one compartment of a Dianorm equilibrium dialyser (Diachema, Langnau am Albis, Switzerland), and dialysed against 1 ml isotonic PBS buffer, pH 7.4. The dialysis time was 16 hr at a temperature of 20 to 22°C. Suramin concentration in the PBS compartment and in the culture medium were determined by high-performance liquid chromatography (HPLC) using a modification of the method described by Teirlynk *et al.* (1989). Briefly, 200-µl samples were mixed with 100 µl Trypan blue as

the internal standard and were de-proteinated with 750 µl of a mixture (53:5:20) of methanol, acetonitrile and TBAHS buffer (0.005 M tetrabutylammoniumhydrogen sulphate and 0.05 M sodium dihydrogen phosphate, pH 6.5 in water) at 4°C. The HPLC analysis was performed using a Nova-Pak C18 Radial-Pak column with a C18 Guard-Pak precolumn (Waters, Milford, CA). Isocratic elution was performed with methanol-acetonitrile-TBAHS buffer (530:75:450) at a flow of 1.5 ml/min. Detection was performed with a variable wavelength detector (model Waters, LambdaMax 481) set at 238 nm. The detection limit was 0.6 µM and the assay was linear between 0.6 and 300 µM suramin.

The amount of protein present in the culture medium was determined by a dye-binding assay (protein assay, BioRad, Munich, Germany).

RESULTS

Initially growth inhibition was tested in standard culture conditions with 5% non-dialysed FCS, which enabled all cell lines to grow exponentially for 7 days using the plating densities described in "Material and Methods". Growth of cells and growth inhibition produced by suramin were determined at different time points after 2, 4 and 6 days exposure. Growth inhibition was dependent on the number of days that cells were in culture, as illustrated for PC-3 cells in Figure 1. This cell line appeared to have the most pronounced time dependent growth inhibition. Figure 2 summarizes the influence of time of culture on IC₅₀ values. Longer exposure clearly enhanced the sensitivity in all cell lines. At exposure times longer than 4 days, only moderate differences in IC₅₀ were observed, compared with 4 days exposure, except for PC3 and HT29 cells.

Exposure for 1 day followed by 5 days recovery in drug-free medium increased the IC₅₀ values for suramin to > 1.5 mM in PC3, MCF-7/ADR^R, HT29 and UM-SCC-11B cells (Table I). In all these cell lines except MCF-7, one day was not enough to retain the inhibitory effect of suramin. After exposure for 3 days followed by 3 days recovery, we obtained IC₅₀ values below 200 µM in MCF-7, SW1573/1R500 and HT29 cells. There was an apparent threshold effect, in that a certain period of exposure was required before the growth inhibition of suramin came into effect. This usually occurred after 1 to 3 days of exposure, except for PC3 cells, where the threshold was after > 3 days of exposure. Longer exposure times added only a little more to growth inhibition. Continuous exposure for 6 days without changing the medium was chosen for subsequent experiments.

FIGURE 1 – Time dependency of growth inhibition of the PC3 cell line by suramin in one representative experiment. PC3 cells were exposed for 2 (+), 4 (▲) or 6 days (■) at different concentrations of suramin. Values are means ± SEM of triplicates.

The effect of different FCS concentrations was studied in all cell lines. The growth of the cell lines was determined in medium with FCS concentrations varying between 0% and 10%. We did not observe large differences in the growth at FCS concentrations between 2.5% and 10% (Fig. 3). Although the SRB test produced high backgrounds in cultures with 10% FCS, the increase in OD could be measured reliably; also, because the difference became larger during culture. However, the background with 10% FCS or 5% FCS plus HSA was too high for reliable determination of growth inhibition, especially at high suramin concentrations (see below). In all cell lines, a dependency of IC_{50} values on the concentration of FCS was

FIGURE 2 – The time dependency of growth inhibition by suramin using continuous exposure. The IC_{50} values determined at 2, 4 and 6 days are represented by open, striped and black bars respectively. Values are means $\pm$ SEM of at least 3 different experiments.

TABLE I – EFFECT OF EXPOSURE AND RECOVERY ON THE GROWTH INHIBITION BY SURAMIN

	1 day	3 days	6 days
PC3	> 1500	755 $\pm$ 40	153 $\pm$ 23
MCF-7	168 $\pm$ 15	59 $\pm$ 9	57 $\pm$ 10
MCF-7/ADR ^R	> 1500	225 $\pm$ 25	86 $\pm$ 20
SW-1573/1R500	502 $\pm$ 32	100 $\pm$ 21	45 $\pm$ 13
UM-SCC-11B	> 1500	320 $\pm$ 25	93 $\pm$ 20
HT 29	> 1500	130 $\pm$ 20	79 $\pm$ 15

Values (in μ M) represent the IC_{50} and are means $\pm$ SEM of at least 3 different experiments. The cells were exposed to suramin for 1, 3 or 6 days (continuous exposure). After exposure for 1 or 3 days the medium was changed for drug-free medium to allow recovery until the end of the experiment.

FIGURE 3 – Growth curves of MCF-7 cells at different FCS concentrations in the culture medium, 0.1% (■), 0.5% (▼), 1% (◆), 2.5% (▲), 5% (+) and 10% (○) FCS. The cells were plated on day 0 as described in "Material and Methods", and the plates were read on days 1, 3, 5 and 7. Values represent the means of 6 wells.

observed (Table II) and the IC_{50} values were markedly lower at 0.5% and 1% FCS than at 5% FCS. PC3 remained the least sensitive cell line. This suggested that the FCS contains factors able to antagonize the effect of suramin. These factors may include protein binding and growth factors present in the FCS.

Because of the large differences between PC3 and MCF-7, these cell lines were chosen for investigation of the factors in FCS that inhibit suramin activity. The influence of the protein binding of suramin on IC_{50} values was investigated by adding HSA to the culture medium. Cells were cultured in the presence of various concentrations of suramin with 1%, 5% and 10% of FCS, and after addition of 1.2 mg of HSA to 1% FCS and 1.3 mg of HSA to medium containing 5% FCS resulting in total protein contents identical to that in 5% and 10% FCS alone respectively. For these experiments the MTT assay was used, since at high protein concentration the background of SRB assay appeared to be too high and interfered with the reproducibility of the experiments. At lower serum concentration, the effects as measured with the SRB and MTT were similar, as has been observed for other agents (Keepers *et al.*, 1991). The IC_{50} values were similar at equal protein concentrations in the medium, and were independent of the amount of FCS present (Table III). These experiments revealed that the most significant factor interfering with the growth inhibition caused by suramin was the amount of protein present in the culture medium. They also suggested that it was not the total amount of suramin added that determines growth inhibition, but the actual concentration of free non-protein-bound suramin. We therefore measured free suramin concentrations in the culture medium. The

TABLE II – SURAMIN CONCENTRATIONS REQUIRED TO PRODUCE 50% OF GROWTH INHIBITION IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF FCS IN THE CULTURE MEDIUM

	0.5% FCS	1% FCS	5% FCS
PC3	11 $\pm$ 5	30 $\pm$ 5	153 $\pm$ 23
MCF-7	4 $\pm$ 2	7 $\pm$ 2	57 $\pm$ 10
MCF-7/ADR ^R	6 $\pm$ 2	23 $\pm$ 5	86 $\pm$ 20
SW-1573/1R500	3 $\pm$ 1	15 $\pm$ 6	45 $\pm$ 13
HT29	3 $\pm$ 1	10 $\pm$ 3	79 $\pm$ 15
UM-SCC-11B	5 $\pm$ 2	11 $\pm$ 1	93 $\pm$ 20

Values (in μ M) represent the mean of the IC_{50} values $\pm$ SEM of at least 3 different experiments on the 6 cell lines at 3 different FCS concentration in the medium after 144 hr of continuous exposure.

TABLE III – EFFECT OF ADDITION OF ALBUMIN ON THE GROWTH INHIBITION BY SURAMIN

	Total suramin		Free suramin	
	MCF-7	PC3	MCF-7	PC3
1% FCS	9 $\pm$ 2	17 $\pm$ 5	6	11
1% FCS + HSA	54 $\pm$ 7	76 $\pm$ 2	12	13
5% FCS	50 $\pm$ 10	88 $\pm$ 9	19	44
5% FCS + HSA	106 $\pm$ 29	180 $\pm$ 15	25	57
10% FCS	105 $\pm$ 29	182 $\pm$ 10	30	69

Values (in μ M) of total suramin represent the mean of the IC_{50} values $\pm$ SEM of 4 different experiments, in medium with 1% FCS (total protein concentration, TP, was 0.3 mg/ml); in 1% FCS + HSA (TP = 1.5 mg/ml); in 5% FCS (TP = 1.5 mg/ml); in 5% FCS + HSA (TP = 3 mg/ml) and 10% FCS (TP = 3 mg/ml). IC_{50} values for free suramin were calculated by correction for bound suramin under the various conditions. In these experiments the MTT assay was used, since the presence of an additional source of protein (HSA) in the culture medium combined with 5% FCS increased the background considerably, reducing the accuracy of the assay. Although the SRB and MTT produced similar IC_{50} values at low serum concentrations, we used the MTT assay for this specific set of experiments.

percentage of free suramin varied from 9% in medium with 10% FCS or 5% FCS plus 1.3 mg/ml of HSA (both having the same amount of proteins) to 86% in 1% FCS medium (Fig. 4). The amount of suramin bound to protein was dependent on the suramin concentration. At a fixed concentration of 5% FCS, the free suramin varied between 20% and 59% at 15 μ M and 150 μ M respectively. These data were used to recalculate the IC_{50} values by correction for the amount of bound suramin (Table III). From these data it was concluded that the protein content of the culture medium is the main factor responsible for the difference in IC_{50} values between cells cultured in 1% and 5% FCS.

Other factors, however, might be present in the FCS that decrease the activity of suramin. Apart from proteins, FCS provides other nutrients to the cells in culture. One of them might be EGF, an important growth factor for both MCF-7 and PC3 cells. Medium supplemented with 1%, 5% FCS and in 5% of dialysed FCS the effect of EGF was, therefore, studied. Dialysed serum and a low concentration of FCS were used to minimize growth stimulation by endogenous growth factors. The addition of EGF at 1 and 10 ng/ml did not affect the growth of the PC3 or MCF-7 cell lines, nor the growth-inhibitory effect of suramin at 1% FCS, 5% undialysed FCS and 5% dialysed FCS in MCF-7 and PC3 cells (Table IV).

In order to further minimize the interference of endogenous growth factors, we tested the ability of PC3 and MCF-7 cells to grow in serum-free medium. For this purpose DME and F-12

were mixed (1:1) and supplemented with ITS without any attachment factor. PC3 cells could be maintained routinely (for at least 6 months) in this serum-free defined medium as described in "Material and Methods", but trypsin had to be neutralized by addition of an equal volume of trypsin inhibitor (0.3 mg/ml in HBSS). Unfortunately, MCF-7 did not grow in this serum-free defined medium, because the cells did not attach. Even when seeded in DMEM supplemented with 1% or 5% FCS, followed by removal of FCS and change to serum-free medium, cells detached spontaneously. PC3 cells grown in serum-free medium were tested for the possible antagonism of several growth factors with the growth-inhibitory effect of suramin. Growth of PC3 cells was not affected by the addition of EGF (5 ng/ml and 25 ng/ml), PDGF (1 ng/ml and 5 ng/ml) and FGF (20 ng/ml and 100 ng/ml) to the defined serum-free medium, nor was the effect of suramin significantly affected by these growth factors (Fig. 5). PDGF even tended to enhance the inhibitory effect of suramin.

DISCUSSION

Suramin has demonstrated anti-tumour activity in various human cancers resistant to conventional chemotherapy, such as prostate carcinoma and adrenocortical tumours. The optimal schedule producing anti-tumour responses, whilst minimizing toxicity, has not been defined. One study comprising 15 patients with various malignancies suggested a correlation between tumour response and suramin plasma levels of about 200 μ M (Stein *et al.*, 1989). These concentrations, however, produce considerable toxicity if maintained for 8 weeks or more, which limits the clinical use of suramin. We have shown that suramin is able to inhibit the growth of a variety of human cancer cell lines (breast, prostate, colon, lung and larynx, 2 of them with a multidrug-resistant phenotype) at concentrations clinically achievable in humans. This growth inhibition is dependent on cell line, concentration and time, in accordance with the literature (LaRocca *et al.*, 1990). In our system, a minimum of 4 days of exposure to suramin seemed to be required to reach IC_{50} values below 200 μ M, except for MCF-7 and SW-1573/1R500 cells (Fig. 2). With longer exposures, IC_{50} values were reached at lower suramin concentrations, although the difference between 4 and 6 days of exposure was considerable less than with shorter exposure times. The experimental approach we used limited the period of culture to 6 days. It is, therefore, still conceivable that exposure times

FIGURE 4 – Suramin binding to serum proteins and HSA. The percentage of free suramin present in different culture mediums is shown. Three different concentrations of the drug have been used. (a) Culture medium supplemented with 1% FCS; (b) with 5% FCS; (c) with 1% FCS plus 1.2 mg/ml HSA; (d) with 10% FCS; and (e) with 5% FCS plus 1.3 mg/ml HSA.

TABLE IV – EFFECT OF EGF ADDITION IN THE MEDIUM ON THE GROWTH INHIBITION BY SURAMIN

	MCF-7	PC3
1% FCS	9 ± 1.8	33 ± 7
1% FCS + 1 ng/ml EGF	10 ± 1.6	38 ± 7
1% FCS + 10 ng/ml EGF	11 ± 0.9	42 ± 9
5% FCS	52 ± 5	145 ± 9
5% FCS + 1 ng/ml EGF	67 ± 5	157 ± 5
5% FCS + 10 ng/ml EGF	72 ± 4	175 ± 9
5% FCS (D) ¹	43 ± 10	103 ± 17
5% FCS (D) ¹ + 1 ng/ml EGF	44 ± 10	93 ± 12
5% FCS (D) ¹ + 10 ng/ml EGF	45 ± 11	124 ± 26

The EGF concentration represents the final concentration in the culture medium. –¹(D) represents dialysed FCS. –Values represent the mean of the IC_{50} values ± SEM on PC3 and MCF-7 cell lines from 4 different experiments, after 6 days of continuous exposure. Values (in μ M) of corresponding experiments were not significantly different, as determined by Student's *t*-test.

FIGURE 5 – Growth inhibition curves of PC3 cells cultured in serum-free medium after 6 days of continuous exposure to suramin alone (+) or with 5 ng/ml of PDGF (▲), 100 ng/ml of FGF (◆) or 25 ng/ml of EGF (○) present in the culture medium (DME/F-12). Points represent means ± SEM of 4 different experiments.

of many weeks, such as can be established in patients treated with suramin, increases the anti-tumour activity of the drug. The short exposure to a high peak value (as might occur with intermittent treatment) is unlikely to have an additional anti-tumour effect *in vivo*.

We observed that the FCS concentration which provides the culture medium with excess nutrients (hormones, growth factors and other substances) has a real impact in the action of suramin in all 6 cell lines tested, decreasing the IC_{50} values more than 10 times when the FCS concentration decreased from 5% to 0.5%. It has been demonstrated that suramin is 99.7% bound to plasma proteins ($\pm 80\%$ to albumin) and does not seem to be metabolized *in vivo* or *in vitro* (Collins *et al.*, 1986). We have demonstrated that in our *in vitro* conditions the main antagonistic effect on growth inhibition by suramin was the albumin present in FCS. These observations could mean that free suramin is indeed required for its antiproliferative activity. Pollak and Richard (1990) also observed an inhibition by albumin on the action of suramin in osteogenic sarcoma cells. These observations indicate that clinical pharmacokinetic studies with free and bound suramin should be performed to determine whether free or bound suramin is responsible *in vivo* for the anti-tumour activity and/or the toxicity. It seems likely that, in patients who have lowered serum albumin concentrations, special care should be taken to prevent high peak levels of suramin, as not only the anti-tumour activity of suramin but also its toxicity may be increased in such conditions. Analogues of suramin with lower protein binding might have better prospects.

The mechanism by which suramin exerts its anti-tumour activity is unclear. It has been demonstrated that suramin is able to inhibit a large number of intra- and extracellular systems *in vitro*. These include cellular enzymes required for cell maintenance and proliferation such as DNA polymerases (Jindal *et al.*, 1990), protein kinase C (Hensey *et al.*, 1989) and laminin (Zabrenetzky *et al.*, 1990). In addition, suramin binds to a variety of growth factors which might play an important role in the biology of cancer cell lines and normal cell lines as well (Pollak and Richard, 1990). It has been demonstrated that suramin counteracts the growth-stimulating effects of andro-

gen, PDGF, FGF and EGF in androgen-responsive cell lines (Berns *et al.*, 1990). In PC3 and MCF-7 cells, the addition of EGF at different FCS concentrations produced only marginal differences in the growth or in the growth inhibition by suramin. In PC3 cells cultured in serum-free medium, EGF, PDGF and FGF showed similar results. LaRocca *et al.* (1991), using a different experimental approach, obtained the same results in PC3 cells and they postulated that the inhibitory properties of suramin result from interaction with an unknown growth factor common to all prostate cancer cells or from inactivation of intracellular enzyme activity. In normal, benign hyperplastic and malignant prostate cells from primary tumours cultured in serum-free medium, Peehl *et al.* (1991) did not observe an appreciable antagonistic effect on the cytostatic activity of suramin with 10-fold excess of EGF, pituitary extract and insulin, alone or in combination. These growth factors may not play an important role in these 2 cell lines in our culture conditions, even at low FCS concentrations. PC3 cells cultured in serum-free medium may be able to produce sufficient autocrine growth factors, and it might be possible that these growth factors are only marginal requirements for the cell biology of our cell lines, and thus do not affect the growth inhibition by suramin.

In conclusion, suramin was able to inhibit the proliferation of 6 human cancer cell lines from different histological origins in a concentration- and time-dependent fashion. The effect of suramin was observed at concentrations that could be achieved in clinical trials. These inhibitory effects are very highly dependent on the free suramin concentration.

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