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Antitumour activity of suramin analogues in human tumour cell lines and primary cultures of tumour cells from patients

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Abstract

Suramin has shown promising antitumour activity against several tumour types, both *in vitro* and *in vivo*, but the clinical utility of this compound is hampered by its unfavourable toxicity profile. In the present study, the semi-automated fluorometric microculture cytotoxicity assay (FMCA) was employed for evaluation of the cytotoxicity of seven suramin analogues *in vitro* in a panel of human tumour cell lines and in primary cultures of tumour cells from patients. Like suramin, the analogues showed little sensitivity to resistance mechanisms involving P-glycoprotein, topoisomerase II, multidrug resistance associated protein and glutathione-mediated drug resistance. In the cell line panel, NF067 and FCE 26644 showed activity comparable with suramin. All analogues were less potent than suramin in patient cells except for FCE 26644. Correlation to suramin activity patterns in the cell line panel was highest for NF037 and low to moderate for the remaining analogues. In patient cells, high correlation coefficients were obtained for FCE 26644, NF110, NF031 and NF037. The results indicate that the cytotoxic activity of suramin on patient tumour cells is shared by the analogues with FCE 26644 being the most active. The pharmacophore for cytotoxicity in patient cells may be different from that observed in the cell lines.

Keywords: Suramin analogue; Tumour cell; *In vitro*; Cytotoxicity

1. Introduction

Suramin is a polysulphonated naphthylamine derivative of urea which was synthesised at the beginning of this century and subsequently employed clinically in the treatment of trypanosomiasis and other parasitic diseases. It exerts part of its trypanocidal activity through the inhibition of the cytosolic serine oligopeptidase, called OP-Tb [1,2]. More recently, suramin has been shown to have activity against HIV-1, which led to clinical trials in patients with AIDS. Although, the activity of suramin against AIDS is not impressive, yet, it exhibits both adrenolytic and antitumour properties [1]. These findings prompted studies of suramin against adrenocortical carcinoma and other malignant tumours. Furthermore, suramin has activity against several metastatic carcinomas refractory to conventional chemotherapy, including those of the kidney, prostate, ovary, oesophagus and adrenal cortex, as well as against non-Hodgkin's lymphoma [3–5]. However, the clinical role of suramin as an antitumour agent is not yet defined and potentially life-threatening toxicity has been observed in several clinical trials. Toxicity seems to be related to high plasma concentrations, and as a result, monitoring of plasma suramin concentration has become an integral part of therapy [3]. The major problem with suramin has been the narrow therapeutic index. However, recent studies have shown that toxicity might be reduced by using suramin in combination with chemotherapy, thereby allowing reduced doses of suramin making therapy more feasible [6,7].

The mechanism of the antitumour activity of suramin is still unknown but the compound may inhibit the interaction between growth factors and their receptors, protein tyrosine-phosphatases and angiogenesis [3,4,8–14].

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In a previous study, the activity of suramin in primary cultures of tumour cells from patients using the fluorometric microculture cytotoxicity assay (FMCA) has been investigated [15]. Results of this study suggested suramin shows a unique activity pattern, being more active against some very resistant solid tumours than against generally chemosensitive haematological tumours. Given the apparent non-cross resistance with other mechanistic classes of cytotoxic agents, it seems worthwhile to identify the suramin pharmacophore for the future design of new suramin derivatives with improved therapeutic ratios. Therefore, we investigated some suramin analogues for their cytotoxic activity against both primary cultures of human tumour cells and established cell lines.

2. Materials and methods

2.1. Cell lines

A human cell line panel of four sensitive parental cell lines, five drug-resistant sub-lines, representing different mechanisms of resistance, and one cell line with primary resistance was used. The cell lines included were the myeloma cell line RPMI 8226/S and its sub-lines 8226/Dox40 and 8226/LR-5 (kind gifts from W.S. Dalton, Department of Medicine, Arizona Cancer Center, University of Arizona, Tucson, AZ, USA), the lymphoma cell lines U-937 GTB and its sub-line U-937-Vcr [16,17], the small cell lung cancer (SCLC) cell line NCI-H69 and its sub-line H69AR (American Type Culture Collection; ATCC, Rockville, MD, USA), the renal adenocarcinoma cell line ACHN (ATCC) and the leukaemic cell line CCRF-CEM and its subline CEM/VM-1 (kind gifts from W.T. Beck, Department of Pharmacology, College of Medicine, University of Tennessee, Memphis, TN, USA). The 8226/Dox40 was selected for doxorubicin (Dox) resistance and shows the classical multidrug resistance (MDR) phenotype with overexpression of P-glycoprotein (Pgp; [18]). The 8226/LR-5 was selected for melphalan resistance, proposed to be associated with increased levels of glutathione (GSH: [19,20]). U-937-Vcr was selected for vincristine (Vcr) resistance, proposed to be tubulin associated [17]. The H69AR, selected for doxorubicin (Dox) resistance, expresses a multidrug resistant (MDR) phenotype proposed to be mediated by a multidrug resistance associated protein (MRP; [21,22]). The CEM/VM-1, selected for teniposide resistance, expresses an atypical MDR, which is proposed to be topoisomerase II (topoII)-associated [23,24]. The exact mechanism of resistance for the primary resistant ACHN cell line is not known and may be multifactorial [25]. The cell lines were cultured as previously described [26].

2.2. Patient samples

A total of 19 patient tumour samples from the different diagnoses were used to determine the activity of suramin analogues. The tumour samples were obtained by bone marrow/peripheral blood sampling, routine surgery or diagnostic biopsy, and this sampling has been approved by the local ethical committee at the Uppsala University Hospital. Leukaemic cells were isolated from bone marrow or peripheral blood by 1.077 g/ml Ficoll-Paque (Pharmacia-Upjohn, Uppsala, Sweden) density gradient centrifugation [27]. Tumour tissue from solid tumour samples was minced into small pieces and the cells were then isolated by collagenase dispersion followed by Percoll (Pharmacia-Upjohn) density gradient centrifugation [28]. Cell viability was determined by trypan blue exclusion test and the proportion of tumour cells in the preparation was judged by inspection of May–Grünwald–Giemsa stained cytospin preparations by a cytopathologist. In some cases, cells were cryopreserved in 10% dimethylsulphoxide (DMSO; Sigma Chemical Co., St Louis, MO, USA) in inactivated fetal calf serum (FCS; HyClone, Cramlington, UK) by initial freezing for 24 h at −70°C, followed by storage in liquid nitrogen. Cryopreservation in this way does not affect drug sensitivity [29].

2.3. Reagents and drugs

Fluorescein diacetate (FDA; Sigma) was dissolved in DMSO and kept frozen (−20°C) as a stock solution (10 mg/ml) protected from light. Culture medium RPMI-1640 (HyClone, Cramlington, UK) supplemented with 10% inactivated FCS, 2 mM L-glutamine, 50 μg/ml of streptomycin and 60 μg/ml of penicillin was used throughout for both cell lines and patient samples. Suramin was obtained from Sigma and FCE 26644 was a kind gift from Farmitalia, Italy. The NF series of analogues (Table 1) were provided by P. Nickel. All analogues were dissolved in sterile water. Dox and Vcr were obtained from commercial sources and were dissolved according to guidelines from the manufacturer and further diluted in phosphate buffer saline (PBS; HyClone) or sterile water.

In the cell line panel, suramin and its analogues were tested twice at five different drug concentrations, obtained by 5-fold serial dilution from an initial concentration of 1000 μg/ml. Five different drug concentrations obtained by a 5-fold serial dilution of the drugs from 200 μg/ml were used in patient samples. Comparison between patient samples was done at 200 μg/ml.

Ninety-six-well microtitre plates (Nunc, Roskilde, Denmark) were prepared with 20 μl/well of drug solution at 10 times the desired concentration, with the aid of a programmable pipetting robot (Propette, Perkin

Elmer, Norwalk, CT, USA). The plates were stored frozen at −70°C for up to 2 months until further use. Under these conditions, no apparent change in drug activity was observed [27].

2.4. The fluorometric microculture cytotoxicity assay procedure (FMCA)

The FMCA is based on the measurement of fluorescence generated from the hydrolysis of fluorescein diacetate (FDA) to fluorescein by cells with intact plasma membranes and has been previously described in detail [27]. Briefly, the cells were resuspended in complete medium, and 180 μl cell suspension was seeded into the wells of 96-well experimental microtitre plates prepared with drugs as described above. Cell densities were 5000–20 000 cells/well for the cell lines, 10 000–20 000 cells/well for the solid tumour cells and 50 000–100 000 cells/well for the haematological tumour cells. Each drug and concentration was tested in triplicate. Six wells with cells but without drugs served as controls and six wells with only culture medium as blanks.

The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 72 h. At the end of the incubation period the plates were centrifuged (1000 rpm, 5 min) and the medium was removed by aspiration. After one wash in PBS, 100 μl/well of FDA (10 μg/ml) dissolved in physiological buffer was added. The plates were incubated for 30 min and the generated fluorescence from each well was measured at 538 nm in a 96-well scanning fluorometer (Fluoroscan II, Labsystems Oy, Helsinki, Finland). The fluorescence is proportional to the number of intact cells in the well.

2.5. Quality control

Quality criteria for a successful analysis included a fluorescence signal in the control wells of more than five times the mean blank value, a mean coefficient of variation (CV) in the control wells of less than 30%, and a tumour cell proportion of more than 70%, in the cell preparation prior to incubation and/or at day 3.

2.6. Quantification of FMCA results

Cell survival is presented as a survival index (SI), defined as the fluorescence in the experimental wells in per cent compared with that in control wells, with blank values subtracted. The IC_{30} was defined as the concentration giving a SI of 70%. When a SI of 70% was not reached the highest concentration, 1000 μg/ml was used as IC_{30}.

For the cell lines the IC_{30}s were evaluated for each individual cell line and drug with custom-made computer software [26]. A delta value for each drug and cell line was calculated as the logarithm of the IC_{30} of the individual cell line minus the mean of all 10 log IC_{30}s [30]. The resistance factors (RFs) in each sub-line were defined as the IC_{30} of the resistant sub-line divided by the IC_{30} of its sensitive parental cell line. Correlation coefficients were determined using Pearson's correlation coefficient.

3. Results

Two of the suramin analogues, NF036 and NF033 are asymmetric whereas suramin and the remaining five analogues are symmetric molecules as shown in Fig. 1. In Fig. 2(a) the concentration–response curves for suramin in the cell line panel are shown together with the resulting mean graph pattern (Fig. 2b). The mean graph panel shows that the small-cell cancer cell line NCI-H69 and its resistant subtype were highly resistant. However, ACHN exhibited slight resistance as compared with the mean. RPMI 8226/Dox40 and RPMI 8226/LR-5 were considerably more sensitive than the average, followed by U-937-GTB and its resistant phenotype, U-937-Vcr.

In the cell line panel, NF067 and FCE 26644 showed comparable activity to suramin with the mean IC_{30}s of 212.3, 298.2 and 246.9 μg/ml, respectively, whereas the other analogues were less active. However, the IC_{30}s varied considerably amongst the cell lines for both suramin and the analogues. The mean SI at 200 μg/ml was lowest for suramin as compared with its analogues (Table 1). Owing to the different molecular weights of the compounds, IC_{30}s were also expressed in μM concentrations. Expressed in this way NF037 was more comparable with suramin than NF067 (Table 1).

Like for suramin, resistance mechanisms involving P-glycoprotein, topoisomerase II, MRP and GSH had minor impact on analogue activity. However, for most of the analogues, the resistance factor for MRP was not

Table 1
Activity of suramin and its analogues in the human tumour cell line panel

Compound	IC_{30}[a] μg/ml (μM) Mean value	SI (200 μg/ml)[b] Mean value
Suramin	246.9 (173)	49.8
NF031	434.1 (310)	65.1
NF033	463.3 (645)	102.9
NF036	561.3 (919)	100.9
NF037	390.5 (279)	58.4
NF067	212.3 (304)	103.1
NF110	362.3 (330)	91.5
FCE 26644	298.2 (253)	67.9

[a] IC_{30} denotes the drug concentration resulting in a survival index (SI) of 70%. SI(%) was calculated: $\frac{\text{mean fluorescence exp. wells}-\text{blank}}{\text{mean fluorescence control wells}-\text{blank}} \times 100$

[b] 200 μg/ml corresponds to 140, 143, 279, 327, 143, 287, 482, 170 μM of suramin, NF031, NF033, NF036, NF037, NF067, NF110 and FCE 26644, respectively.

determined because the IC_{30} could not be achieved in the small-cell lung cancer cell lines reflecting the resistance mechanism in these cells. Interestingly, NF067 and NF031 showed substantial resistance factor of 16.1 and 16.9 for MRP and P-glycoprotein, respectively (Table 2).

In the patient tumour cells, the analogues, with the exception of FCE 26644, were mostly less potent than suramin (Table 3). To get an indication of the similarity of the analogues in their mode of action compared with suramin, correlation coefficients between SI values at 200 μg/ml were calculated. Correlation to suramin activity patterns in the cell line panel was highest for NF037 and low to moderate for the remaining analogues (Table 4). In patients' cells, high correlation coefficients were obtained for FCE 26644, NF110, NF031 and NF037, indicating a similar mode of action (Table 4). The lowest correlations in both cell systems were obtained for the two asymmetrical molecules (NF033 and NF036) and for NF067, which contains two diphosphatylated phenyl rings instead of the polysulphonated napthalenes (Table 4).

4. Discussion

The specific activity of cytotoxic drugs in tumours, in accordance with clinical experience, seems to be detected *in vitro* by non-clonogenic drug resistance assays [31]. Validity of the FMCA procedure has already been proved in describing tumour-type specific activity of standard drugs in both haematological and various solid tumours [15,32,33]. For suramin, the activity pattern as detected by FMCA has been shown to be

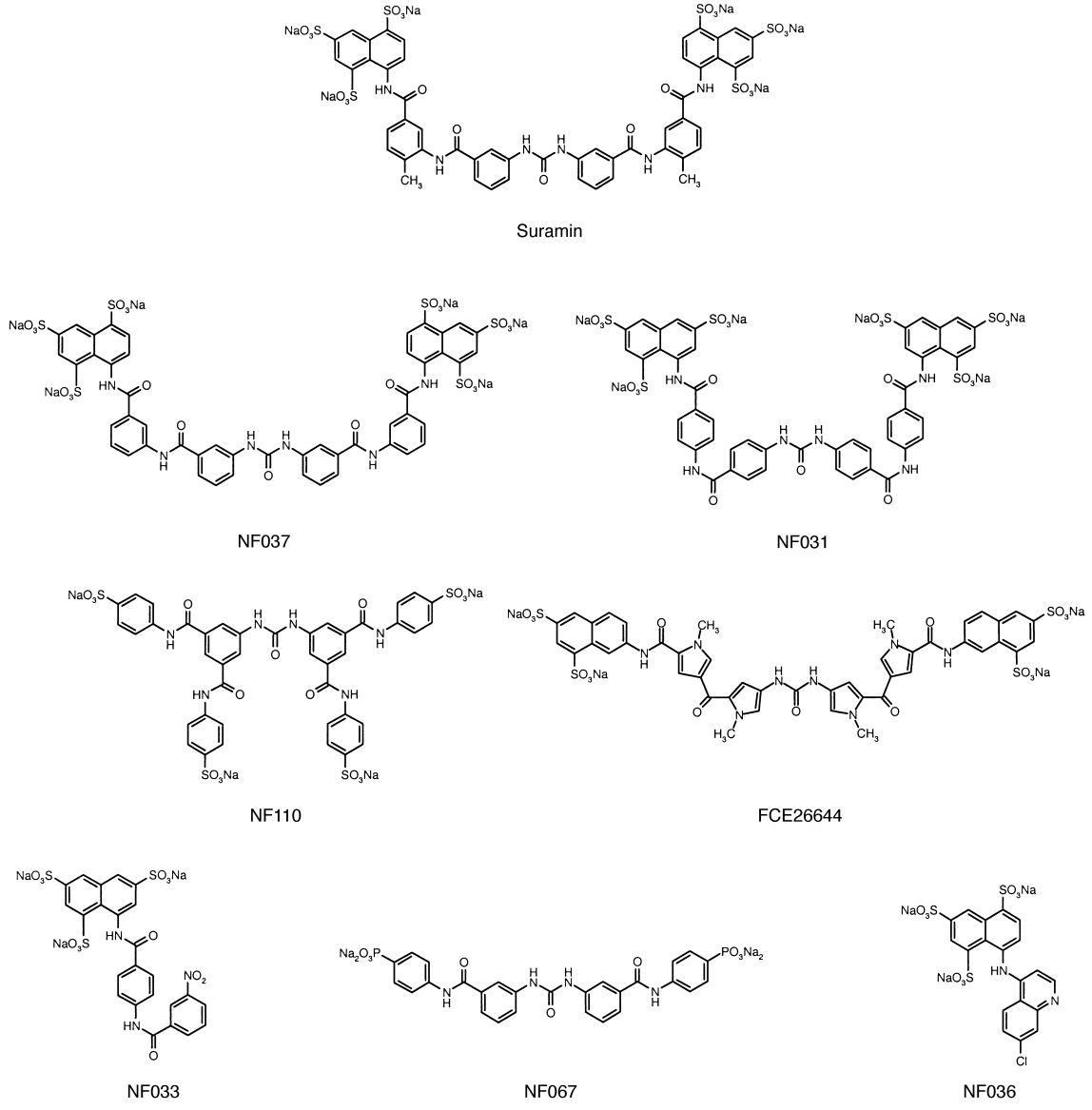

Fig. 1. Chemical structures of the investigated compounds.

Table 2
Resistance factors (RF)[a], in the cell lines representing the indicated resistance mechanisms, for the investigated compounds[b]

Cell lines tested/ Compound	Resistance mechanism				
	RPMI 8226 DOX 40/S/ Pgp	CEA-VMI/CEM/ Topo II	U937-Vcr/GTB/ Tubulin	RPMI 8226-LRI/5/ GSH	H69AR/H69/ MRP
Suramin	0.29	0.87	0.89	0.64	nd
NF031	10.90	0.70	1.08	0.87	nd
NF033	0.22	0.84	0.88	0.56	2.50
NF036	0.21	0.70	nd	2.71	3.52
NF037	4.01	0.21	0.96	1.24	nd
NF067	1.04	0.49	0.50	1.09	16.12
NF110	2.48	0.12	0.92	0.13	nd
FCE 26644	0.06	1.04	2.71	0.31	nd

[a] Resistance factor (RF) = IC_{30} in resistant cell line/IC_{30} in parental cell line.
[b] Pgp, P-glycoprotein associated resistance; Topo II, topoisomerase II associated resistance; Tubulin, Tubulin associated resistance, GSH, glutathione associated resistance; MRP, multidrug associated resistance protein; nd, not determined.

particularly interesting with an observed unprecedented activity in solid tumours, most notably colon carcinoma [15]. However, the adverse effect profile of suramin makes the search for less toxic analogues with retained anticancer activity necessary.

In this study, a panel of 10 human tumour cell lines was used to provide a fingerprint of the differential activity of suramin across a cell panel with defined mechanisms of resistance. Previous studies, using human tumour cell lines as a model system, have shown higher antiproliferative activity for several analogues compared with suramin [34–36]. In the present study, the relative potency of the suramin analogues varied between cell lines, but considering the mean activity of the cell line panel no analogue showed considerably improved activity over suramin. This was also the case in cultures of patient tumour cells. However, some analogues showed comparable potency with suramin and any potential therapeutic benefit will, therefore, depend on their toxicity profile. In this respect, FCE 26644 may be a potentially interesting candidate drug for further clinical development since its toxicity profile in animal models has been found to be favourable compared with suramin [37].

When correlations to suramin activity patterns were used as an indication of a similar mode of action, there was an apparent difference between the cell lines and patient cells. Since, the FMCA measures cytotoxic rather than cytostatic effects in the low proliferative PHTC cultures, these experiments indicate that suramin

Table 3
Effect of suramin and its analogues on survival index at 200 μg/ml in the primary human tumour cell samples from patients[a]

Patient *n*	Diagnosis	Survival index (%)							
		NF031	NF033	NF036	NF037	NF067	NF110	FCE 26644	Suramin
1	Mesothelioma	74	100	93	102	45	89	74	57
2	Ovarian cancer	88	107	97	80	93	87	66	71
3	Colon cancer	54	94	94	53	82	77	32	40
4	Colon cancer	24	92	96	28	89	62	8	10
5	NHL	94	95	90	104	102	91	101	105
6	Colon cancer	18	55	69	40	177	69	15	17
7	NHL	342	158	96	426	222	261	460	381
8	AML	55	87	73	45	58	52	19	35
9	Renal cancer	78	106	89	89	104	95	73	86
10	Colon cancer	19	46	94	22	95	53	10	11
11	Wilms' tumour	123	113	135	140	140	159	153	143
12	Colon cancer	26	103	117	41	102	100	17	32
13	Bladder cancer	nd	nd	nd	nd	nd	nd	25	39
14	AML	113	106	118	48	152	61	29	40
15	Breast cancer	47	79	66	83	nd	nd	55	58
16	Adrenal cancer	nd	nd	nd	63	nd	nd	29	42
17	Renal cancer	44	131	135	51	113	102	25	39
18	Ovarian cancer	nd	nd	nd	85	nd	nd	106	99
19	Ovarian cancer	nd	nd	nd	125	nd	nd	103	95
	Mean value[b]	61.2	97.6	93.9	63.3	101.2	86.6	30.3	40.8
	S.D.	34.7	22.2	21.6	34.2	35.4	28.4	41.7	36.3

[a] NHL, non-Hodgkin's lymphoma; AML, acute myelocytic leukaemia; S.D., standard deviation.
[b] Patient no. 7 showing growth stimulation is not included in this calculation.

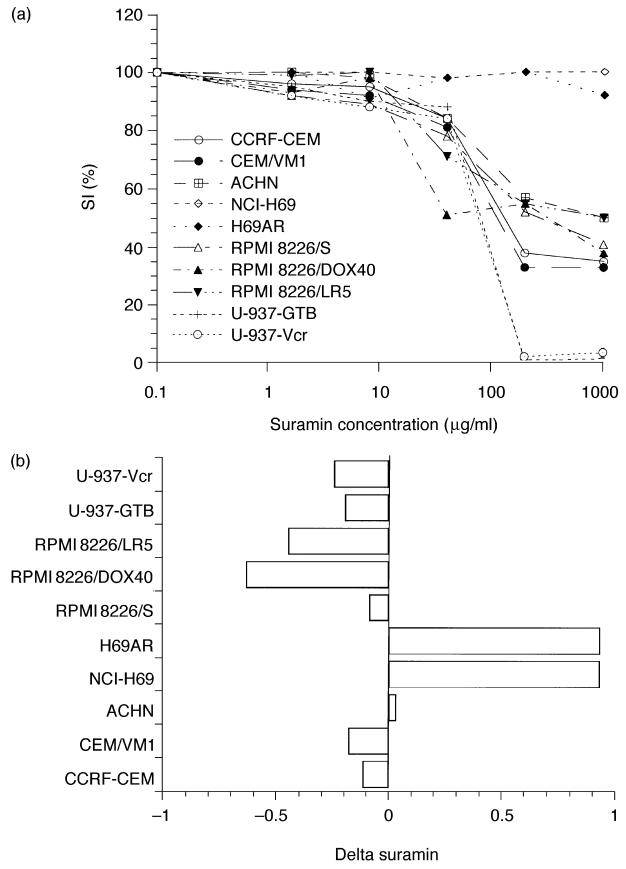

Fig. 2. Effect of suramin on survival index (SI) in all investigated cell lines (a). From these concentration–response curves, mean $\log_{10}$ IC_{30} in the cell lines was calculated. The difference between the $\log_{10}$ of each cell line and the mean $\log_{10}$ IC_{30} was calculated to yield a variable denoted as delta. A mean graph consisting of the drug-specific deltas across the cell line panel could then be constructed to visualise differential cytotoxicity patterns of drugs (b). Bars projecting to the left (negative values) indicate cell lines more sensitive than average and bars projecting to the right (positive values) indicate cell lines more resistant than average for suramin.

and some of the analogues have cytotoxic properties which are unrelated to inhibition of proliferation and that the pharmacophore for cytotoxicity may be different from that for antiproliferation. In patient cell cultures, FCE 26644 showed most similarity to suramin with a correlation coefficient of 0.98. Thus, FCE 26644 appears to be a good candidate for further development in the treatment of cancer.

In one of the samples (non-Hodgkin's lymphoma) there was a clear paradoxical concentration-dependent increase in SI values upon suramin administration. This was actually shared by the majority of the analogues in this sample, as well as in some cell lines, and calls for caution when potential clinical application is considered. This stimulatory effect on cell proliferation might be related to multiple interactions of suramin with signalling pathways which in some cell systems may be linked to the stimulation of cell proliferation rather than to cell death [38–40].

Table 4
Correlations between survival indices (SI) for suramin and the different analogues at 200 μg/ml in the human tumour cell lines and primary human tumour cells from patients

Compound	Cell line panel R^a	Patient cells R
Suramin	1.0	1.00
NF031	0.13	0.81
NF033	0.31	0.47
NF036	0.34	0.28
NF037	0.94	0.93
NF067	0.19	0.09
NF110	0.45	0.80
FCE 26644	0.48	0.98

[a] Pearson's correlation coefficient.

Any structure–activity relationship for the cytotoxic effect was difficult to discern due to the relatively few analogues tested. However, as in a previous study of antiproliferative effects [36] only symmetrical derivatives of urea showed activity and similarity to suramin. Therefore, the prerequisite for suramin-like activity seems to be a large symmetrical molecule (urea derivative) with polysulphonated napthalene moieties.

In view of the findings that the suramin activity is different from that of conventional cytotoxic agents, standard drug and suramin combinations should be investigated further. The interaction of low-dose suramin with paclitaxel and possible synergistic effects of the drug in combination with other antitumour agents on human cell lines clearly have potential benefits [7,41,42].

In summary, the results suggest that the pharmacophore for cytotoxicity in patient tumour cells may be different from that of the antiproliferative effects observed in cell lines. Furthermore, the results indicate that the specific cytotoxic activity of suramin on tumour cells from patients is shared by some symmetrical analogues, FCE 26644 being the most active and suitable for further development as an anticancer drug.

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References

1. Stein C. Suramin: a novel antineoplastic agent with multiple potential mechanisms of action. *Cancer Res* 1993, **53**, 2239–2248.

2. Morty R, Troeberg L, Pike R, *et al*. A trypanosome oligopeptidase as a target for the trypanocidal agents pentamidine, diminazene and suramin. *FEBS Lett* 1998, **433**, 251–256.
3. Scher H, Kelly W. Suramin: defining the role in the clinic. *Princ Pract Oncol Updates* 1993, **7**, 1–16.
4. Voogd T, Vansterkenburg E, Wilting J, Janssen L. Recent research on the biological activity of suramin. *Pharmacol Rev* 1993, **45**, 177–203.
5. Shin R, Naomoto Y, Kamikawa Y, Tanaka N, Orita K. Effect of suramin on human esophageal cancer cells *in vitro* and *in vivo*. *Scand J Gastroenterol* 1997, **32**, 824–828.
6. Frommel T. Suramin is synergistic with vinblastine in human colonic tumour cell lines: effect of cell density and timing of drug delivery. *Anticancer Res* 1997, **17**(3C), 2065–2071.
7. Miglietta L, Canobbio L, Granetto C, Vannozzi M, Esposito M, Boccardo F. Suramin/epidoxorubicin association in hormone-refractory prostate cancer: preliminary results of a pilot phase II study. *J Cancer Res Clin Oncol* 1997, **123**(7), 407–410.
8. Minniti C, Maggi M, Helman L. Suramin inhibits the growth of human rhabdomyosarcoma by interrupting the insulin-like growth factor II autocrine growth loop. *Cancer Res* 1992, **52**, 1830–1835.
9. Kragh Larsen A. Suramin: an anticancer drug with unique biological effects. *Cancer Chemother Pharmacol* 1993, **32**, 96–98.
10. Fujiuchi S, Ohsaki Y, Kikuchi K. Suramin inhibits the growth of non-small-cell lung cancer cells that express the epidermal growth factor receptor. *Oncology* 1997, **54**(2), 134–140.
11. Gagliardi A, Kassack M, Kreimeyer A, Muller G, Nickel P, Collins D. Antiangiogenic and antiproliferative activity of suramin analogues. *Cancer Chemother Pharmacol* 1998, **41**(2), 117–124.
12. Zhang Y, Keng Y, Zhao Y, Wu L, Zhang Z. Suramin is an active site-directed, reversible, and tight-binding inhibitor of protein-tyrosine phosphatases. *J Biol Chem* 1998, **273**(20), 12281–12287.
13. Benini S, Baldini N, Manara M, *et al*. Redundancy of autocrine loops in human osteosarcoma cells. *Int J Cancer* 1999, **80**, 581–588.
14. Bocci G, Danesi R, Benelli U, *et al*. Inhibitory effect of suramin in rat models of angiogenesis *in vitro* and *in vivo*. *Chemother Pharmacol* 1999, **43**, 205–212.
15. Csoka K, Nygren P, Graf W, Påhlman L, Glimelius B, Larsson R. Selective sensitivity of solid tumors to suramin in primary cultures of tumor cells from patients. *Int J Cancer* 1995, **63**, 356–360.
16. Sundström C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line. *Int J Cancer* 1976, **17**, 565–577.
17. Botling J, Liminga G, Larsson R, Nygren P, Nilsson K. Development of vincristine resistance and increased sensitivity to cyclosporin A and verapamil in the human U-937 lymphoma cell line without over expression of the 170 kDa P-glycoprotein. *Int J Cancer* 1994, **58**, 269–274.
18. Dalton W, Durie B, Alberts D, Gerlach J, Cress A. Characterisation of a new drug-resistant human myeloma cell line that expresses P-glycoprotein. *Cancer Res* 1986, **46**, 5125–5130.
19. Bellamy W, Dalton W, Gleason M, Grogan T, Trent J. Development and characterisation of a melphalan-resistant human multiple myeloma cell line. *Cancer Res* 1991, **51**, 995–1002.
20. Mulcahy R, Bailey H, Gipp J. Upregulation of gamma-glutamylcysteine synthetase activity in melphalan-resistant human multiple myeloma cells expressing increased glutathione levels. *Cancer Chemother Pharmacol* 1994, **34**, 67–71.
21. Mirski S, Gerlach J. Multidrug resistance in a human small cell lung cancer cell line selected in adriamycin. *Cancer Res* 1987, **47**, 2594–2598.
22. Cole S, Bhardway G, Gerlach J, Almquist K, Deelay R. A novel ATP binding cassette transporter gene overexpressed in multidrug resistant human lung tumor cells. *Science* 1992, **268**, 1650–1654.
23. Danks M, Yalowich J, Beck W. A typical multidrug resistance in a human leukemic cell line selected for resistance to teniposide (VM-26). *Cancer Res* 1987, **47**, 1297–1301.
24. Danks M, Schmidt C, Cirtain M, Suttle D, Beck W. Altered catalytic activity of and DNA cleavage by DNA topoisomeras II from human leukemic cells selected for resistance to VM-26. *Biochemistry* 1988, **27**, 8861–8869.
25. Nygren P, Larsson R. Verapamil and cyclosporin A sensitize human kidney tumor cells to vincristine in absence of membrane P-glycoprotein and without apparent changes in the cytoplasmic free Ca^{2+} concentration. *Biosci Rep* 1990, **10**, 231–237.
26. Dhar S, Nygren P, Csoka K, Botling J, Nilsson K, Larsson R. Anti-cancer drug characterisation using a human cell line panel representing defined types of drug resistance. *Br J Cancer* 1996, **74**, 888–896.
27. Larsson R, Kristensen J, Sandberg C, Nygren P. Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia using a fluorometric microculture cytotoxicity assay (FMCA). *Int J Cancer* 1992, **50**, 177–185.
28. Csoka K, Larsson R, Tholander B, Gerdin E, Torre M, Nygren P. Cytotoxic drug sensitivity testing of tumor cells from patients with ovarian carcinoma using the fluorometric cytotoxicity assay (FMCA). *Gynecol Oncol* 1994, **54**, 163–170.
29. Nygren P, Kristensen J, Sundström C, Lönnerholm G, Kreuger A, Larsson R. Feasibility of the fluorometric microculture cytotoxicity assay (FMCA) for cytotoxic drug sensitivity testing of tumor cells from patients with acute lymphoblastic leukemia. *Leukemia* 1992, **6**, 1121–1128.
30. Boyd M, Paull K. Some practical considerations and applications of the National Cancer Institute *in vitro* anticancer drug discovery screen. *Drug Dev Res* 1995, **34**, 91–109.
31. Weisenthal LM, Dill PL, Pearson FC. Effect of prior cancer chemotherapy on human tumor-specific cytotoxicity *in vitro* in response to immunopotentiating biologic response modifiers. *J Natl Cancer Inst* 1991, **83**, 37–42.
32. Nygren P, Hagberg H, Glimelius B, *et al*. In vitro drug sensitivity testing of tumor cells from patients with non-Hodgkin's lymphoma using the fluorometric microculture cytotoxicity assay. *Ann Oncol* 1994, **5**, S127–S131.
33. Nygren P, Fridborg H, Csoka K, *et al*. Detection of tumor specific cytotoxic drug activity *in vitro* using fluorometric microculture cytotoxicity assay and primary cultures of tumor cells from patients. *Int J Cancer* 1994, **56**, 715–720.
34. Jentsch K, Hunsmann G, Hartmann H, Nickel P. Inhibition of human immunodeficiency virus type I reverse transcriptase by suramin-related compounds. *J Gen Virol* 1987, **68**, 2183–2192.
35. Braddock P, Hu D, Fan T-P, Stratford I, Harris A, Bicknell R. A structure-activity analysis of antagonism of the growth factor by suramin and related polyanions. *Br J Cancer* 1994, **69**, 890–898.
36. Firsching A, Nickel P, Mora P, Allolio B. Antiproliferative and angiostatic activity of suramin analogues. *Cancer Res* 1995, **55**, 4957–4961.
37. Ciomei M, Pastori W, Mariani M, Sola F, Grandi M, Mongelli N. New sulfonated distamycin A derivatives with bFGF complex activity. *Biochem Biopharmacol* 1994, **47**(2), 295–302.
38. Vischer TL. Suramin stimulates B-lymphocyte proliferation in the mouse. *Agents Actions* 1976, **6**, 479–482.
39. Foekens J, Sieuwerts A, Stuurman-Smeets E, Dorssers L, Berns E, Klijn J. Pleiotropic actions of suramin on the proliferation of human breast cancer cells *in vitro*. *Int J Cancer* 1992, **51**(3), 439–444.
40. Rubio G, Pinedo H, Virizuela J, van Ark Otte J, Giaccone G. Effects of suramin on human lung cancer cell lines. *Eur J Cancer* 1995, **31A**, 244–251.
41. Andreotti P, Lee J-M, Greenspan E, Bruckner H. Interaction of low-dose suramin with taxol. *Proc ASCO* 1994, **13**, 165.
42. Lopez Lopez R, Van Rijswijk R, Wagstaff J, Pinedo H, Peters G. The synergistic and antagonistic effects of cytotoxic and biological agents on the *in vitro* antitumor effects of suramin. *Eur J Cancer* 1994, **30A**, 1545–1549.