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Preclinical report

Angiostatic effects of suramin analogs *in vitro*

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Suramin analogs are polyanionic naphthylureas structurally related to suramin, an antitumor agent with a narrow therapeutic window. The angiostatic activities of suramin and 16 suramin analogs were investigated using an easily quantifiable *in vitro* angiogenesis system. In addition, the antiproliferative activities of the analogs were studied in four different human tumor cell lines and in porcine aortic endothelial cells. The suramin analogs encompassed two main structural variations, i.e. their molecular size, and the number and substitution pattern of the sulfonate groups. Some suramin analogs with a reduced number of sulfonate groups (NF062, NF289 and NF326) showed significant dose-dependent angiostatic and also antiproliferative activities. The disulfonate NF062 was superior to suramin in inhibiting HT29 and T47D tumor cells while demonstrating a similar angiostatic potential as suramin. Therefore, the sulfonate groups in the *para* position of the amino groups of the naphthyl residues of suramin seem to be of special importance. The very small disulfonates (NF108, NF109, NF499, NF500 and NF241) and the asymmetric compound NF520, one half of the suramin molecule, are inactive. Therefore, a minimal molecule size seems to be essential for the biological activity. Suramin is a rather rigid molecule. The highly flexible analogs (NF527, NF528 and NF529) are inactive. This indicates that the molecular rigidity is important for the biological activity. [© 2000 Lippincott Williams & Wilkins.]

Key words: Microcarrier angiogenesis assay, structure–activity relationship, suramin, suramin analogs.

Introduction

Suramin, a symmetric polyanionic compound (a hexasulfonate with a central urea bridge), was originally used for the treatment of trypanosomiasis and onchocerciasis.[1] Recently it has attracted renewed interest as an anticancer-drug. In clinical trials suramin has been able to disrupt the growth of solid tumors. Efficacy has been reported in advanced cancers of the kidney,[2] adrenal gland,[3–5] ovary[6] and lymphomas.[7] Phase II trials of suramin for metastatic prostate cancer have been completed with promising results.[8–11]

Suramin demonstrates a wide range of activities. *In vitro*, suramin exhibits antiproliferative activity against many tumor cell lines.[12–15] Moreover, suramin has been reported to block the binding of several growth factors including basic fibroblast growth factor,[14,16] epidermal growth factor,[17,18] transforming growth factor-β,[14] platelet-derived growth factor,[19] vascular endothelial growth factor[20] and insulin-like growth factor I[15] to their receptors, respectively, by inducing conformational changes and therefore inhibiting their biological signal. In addition, suramin is a potent inhibitor of a variety of enzymatic systems like protein kinase C,[17,21] topoisomerase II,[22,23] DNA polymerase,[24] ATPase,[25] phosphoinositol kinase, diaceryl kinase[17] and even reverse transcriptases[26] of a number of animal retroviruses. However, despite of the multitude of activities of suramin, the mechanism of action responsible for the antitumor effect *in vivo* remains to be elucidated.

As the use of suramin in humans is limited by severe side effects,[4] new suramin analogs with high antitumor activity and lower toxicity would be of considerable value.

In a previous study we have found that some symmetric suramin analogs inhibited tumor cell growth more potently than suramin.[13] Furthermore we have demonstrated that symmetry of suramin analogs is a prerequisite for antitumor activity. Other studies have demonstrated that some smaller symmetric suramin analogs with a reduced number of sulfonate groups also show high antiproliferative activities and lower toxicities than suramin.[27]

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We and others have also shown that suramin and suramin analogs possess considerable angiostatic activities in the chick chorioallantoic membrane (CAM) assay[13,28] and in other angiogenesis models.[27] As tumor growth is angiogenesis dependent,[29] the angiostatic properties of suramin may be of special importance for its clinical antitumor activity.

Therefore, in this study we have investigated suramin analogs with variations of several structural features. The number of sulfonate groups was varied. Symmetric suramin analogs with two, four or six sulfonate groups (disulfonates, tetrasulfonates and hexasulfonates) were investigated. The molecule size was varied in several ways. Compounds with four benzoyl residues and a central urea bridge (e.g. suramin) are named 'big ureas'. Ureas containing only two benzoyl residues (e.g. NF289) are named 'small ureas'. Most of the investigated compounds are substituted diphenylureas. Urea is a diamide of carbonic acid. In order to clarify if the urea bridge is essential for biological activity, we have replaced the urea bridge by diamides of dicarboxylic acids, here by the diamide of isophthalic acid (e.g. NF674). Suramin is a rather flat and rigid molecule. With the aim to elucidate the importance of this rigidity for biological activity, we prepared flexible suramin analogs by replacing benzoyl residues of suramin by acetyl residues (e.g. NF528) or by phenylacetyl residues (e.g NF529). Finally, we investigated one asymmetric compound (NF520, one half of the suramin molecule).

We compared the ability of suramin analogs to inhibit tumor cell proliferation with their angiostatic efficacy using a recently developed angiogenesis system. In contrast to the CAM assay, this microcarrier (MC) assay[30,31] allows excellent quantification of angiogenesis *in vitro*.

Materials and methods

Materials

Suramin, the hexasodium salt of 8,8′-{carbonylbis[imino-3,1-phenylenecarbonylimino-3,1-(4-methyl-phenylene) carbonylimino] } bis- (1, 3, 5-naphthalenetrisulfonic acid) (Germanin®) was purchased from Bayer (Leverkusen, Germany). Culture media, fetal calf serum (FCS), trypsine–EDTA solution (1 ×), non-essential amino acids (NEAA), phosphate-buffered saline (PBS) and HEPES were obtained from Gibco (Paisley, UK), porcine fibrinogen (fraction I; 93% protein clottable), bovine thrombin, antibiotic–antimycotic solution, hydrocortisone-21-phosphate, insulin transferrin (ITS)-media supplement, L-glutamine, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) and Cytodex 2 microcarriers were obtained from Sigma (St Louis, MO). Collagenase A from *Clostridium histolyticum* was purchased from Boehringer (Mannheim, Germany). Suramin analogs were synthesized at the Pharmaceutical Institute of the University of Bonn, Germany and purity was checked as described previously.[32,33] The chemical structures of the suramin analogs used in the experiments are shown in Figure 1. HT29, PC3, MCF-7 and T-47D cells were obtained from ECACC (Salisbury, UK).

Cell culture

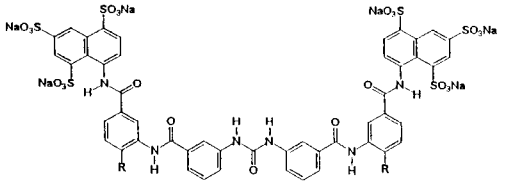
Cells were routinely grown in their respective complete growth media. For HT29 cells: MEM medium containing 10% heat-inactivated FCS, HEPES (20 mM), NEAA (1%), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml) was used. Complete growth medium for routine culture of PC3 cells consisted of HAM's F-12 medium supplemented with 10% FCS and L-glutamine (1%), and for T47D cells RPMI medium supplemented with FCS (10%), porcine insulin (10 μg/ml) and estradiol (1 nM) was used. MCF-7 cells were cultured in MEM containing 10% FCS and NEAA (1%).

Cell proliferation assay

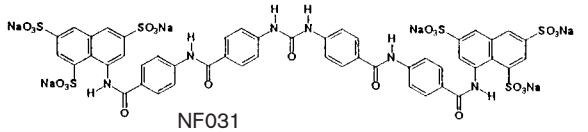
Cells were harvested by trypsination, resuspended in fresh medium in 96-well flat-bottom microtiter plates (Greiner, Solingen, Germany) and incubated in a humidified atmosphere with 5% CO_2 at 37°C in their defined medium in the presence of suramin or suramin analogs (30–500 μM final concentration) for 5 days.

The MTT assay[34] was used to examine the effects of suramin and the various suramin analogs on cell proliferation. IC_{50} values (half maximal inhibitory concentrations) were calculated from cell growth curves for all cell lines and the investigated compounds, respectively. To a 50 μl cell suspension (5000 cells/well), 50 μl test solution was added in individual wells of a 96-well microtiter plate with each replicate for each condition studied. With each suramin analog a minimum of four experiments was evaluated. For the cell lines used in our experiments, we found a linear relation between the results of the MTT assays and the cell numbers in the individual assays. Since the presence of suramin in the medium reduced formazan production in the MTT assay, the wells were washed twice with PBS to remove possible remaining amounts of suramin attached to the cells, before culture medium without drugs, but containing MTT (5 mg/ml), was added. Three hours after the addition the

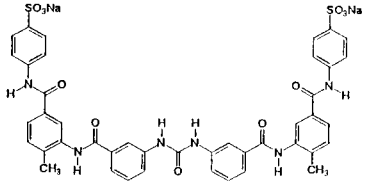
Symmetric compounds (group I)



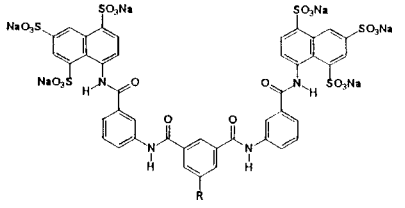
Suramin R=CH_3
NF037 R=H



NF031

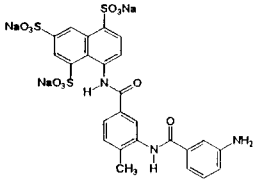


NF062



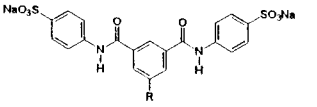
NF674 R=NO_2
NF675 R=NH_2

Asymmetric compound

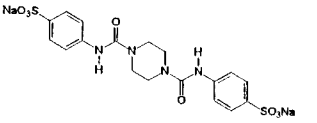


NF520

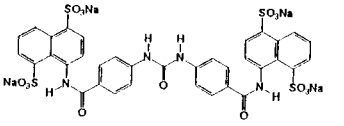
Symmetric compounds (group II)



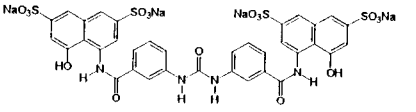
NF108 R=NO_2
NF109 R=NH_2



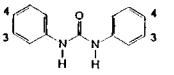
NF241



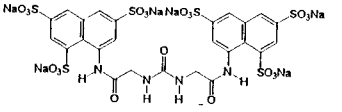
NF289



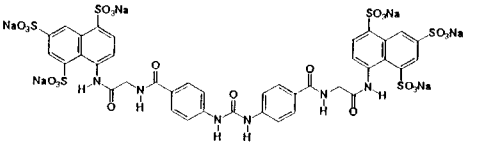
NF326



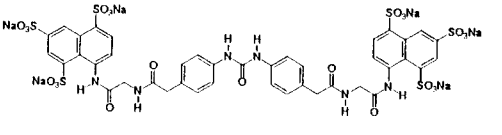
NF499 4-SO_3Na
NF500 3-SO_3Na



NF527



NF528



NF529

Figure 1. The chemical structure of suramin and suramin analogs. Group I: large compounds with an extended multiple ring structure. Group II: compounds of the 'small urea' type.

absorption at 510 nm was measured. Possible remaining amounts of suramin and suramin analogs attached to the cells after washing did not interfere in the MTT assay, as was proven by comparing cell counts and absorbances at 510 nm before and after washing. After incubation the MTT solution was supplemented with 100 μl 0.04 N HCl in isopropranol to dissolve the precipitation and the plates were shaken for 15 min. The optical density at 510 nm was then read on an automatic microplate reader (400 SF; SLT Labinstruments, Grödig, Austria).

MC assay

The ability of the suramin analogs to inhibit angiogenesis was determined using a MC assay as described recently.[30,31]

Porcine aortic arteries were obtained from a local slaughter house, ligated at one end and washed twice with sterile PBS (pH 7.0). Arteries were then filled with collagenase A (0.1% w/v in PBS) and incubated for 8 min at 37°C to detach endothelial cells from the intima. The isolated endothelial cells were suspended in DMEM supplemented with 20% FCS and 100 U/ml antibiotic–antimycotic solution and then centrifuged at 200 *g* for 6 min. After removal of medium, the isolated cells were seeded in gelatine-coated culture flasks (25 cm^2) with fresh medium.

Confluent cells were transferred to large culture flasks (75 cm^2), passaged 4 times and then seeded on MCs (gelatine-coated Cytodex-2 MCs dissolved in PBS). Freshly autoclaved MCs were suspended in PBS and endothelial cells were added to a final concentration of around 30 cells/MC. During a 3 h incubation at 37°C while gently agitating the MCs every 20 min, the cells were allowed to attach to the MCs.

Fibrin gels were prepared as described.[30] Porcine fibrinogen was dissolved in PBS (pH 7.0) and the solution was passed through a 0.2 μm filter. The other day, after adding aprotinin to prevent excess fibrinolysis by fibrin-embedded cells, the fibrinogen solution (1 mg/ml PBS) was transferred to 35 mm culture dishes. Cell-coated MCs were seeded in the fibrinogen solution (about 150 MCs/ml) and thrombin (0.625 U/ml) was added to induce clotting. Thirty minutes later, the gels were equilibrated with DMEM supplemented with 0.2% ITS and antibiotic–antimycotic solution (100 μl/ml). After 1 h equilibration time, fresh medium and the angiostatic drugs were added.

After further incubation, the angiostatic responses were quantified in a blinded manner at day 2, 3 and 4, respectively. The sprouting vessels were focussed with an inverse-microscope through an ocular with a defined counting grid (edge length=100 μm) (magnification: 10×12, $5\times$) after a predefined evaluating scheme. The counting grid was centered on a MC and the number of areas containing sprouting capillaries were counted. In random order, a minimum of 20 MCs was analyzed in each gel and the results of duplicate gels were combined (n=40). For comparison of different experiments, the mean number of areas containing capillaries in control gels at day 4 was set as 100%.

As suramin and suramin analogs may impact on the fibrin matrix we have studied the effects on pH and optical density at 350 nm. No changes in pH value were found. Optical density increased after addition of suramin and suramin analogs but returned to baseline after washing twice with PBS, indicating that no irreversible structural changes occurred.

In addition, cell proliferation with porcine aortic endothelial cells (PAEC) was measured with the MTT assay.

Significance of differences ($p<0.01$) of the angiostatic effects between the suramin analogs and controls, and also between different suramin analogs was calculated by analysis of variance (ANOVA) with *post hoc* analysis by the Neuman–Keuls test using standard statistical software (Statistica; StatSoft, Tulsa, OK).

Results

Structure–activity relationship studies in tumor cells

In a first step, the inhibitory activities of suramin and a series of suramin analogs with varying numbers of sulfonate groups on HT29 cell growth (a colon carcinoma cell line) were investigated. The IC_{50} values obtained for suramin and its analogs are shown in Table 1. Suramin inhibited cell proliferation in a dose-dependent manner with an IC_{50} value of 73 μM. Among the hexasulfonate analogs, only NF674 and NF675 were active (IC_{50} 140 and 198 μM, respec-

Table 1. Antiproliferative activity (IC_{50}[a]) of symmetric suramin analogs in HT29 cells

Hexasulfonates		Tetrasulfonates		Disulfonates	
Compound	IC_{50}	Compound	IC_{50}	Compound	IC_{50}
Suramin	73	NF289	186	NF062	38
NF527	>500	NF326	100	NF108	>500
NF528	>500			NF109	>500
NF529	>500			NF241	>500
NF674	140			NF499	>500
NF675	198			NF500	>500

[a]Concentration (μM) inducing 50% growth inhibition

tively) but significantly less active than suramin. The tetrasulfonate NF326 had an activity (IC_{50} 100 μM) comparable to that of suramin, whereas the tetrasulfonate NF289 (IC_{50} 186 μM) was significantly less active than suramin. Surprisingly, the disulfonate NF062 (IC_{50} 38 μM) was a significantly more potent inhibitor than suramin. In contrast, none of the five other disulfonates showed an inhibitory effect up to a concentration of 500 μM.

Depending on the availability, the compounds were also screened with PC3, T47D, MCF7 and PAEC. The results are shown in Table 2. Suramin showed an antiproliferative activity in all four cell lines with IC_{50} values ranging from 57 to 113 μM. The hexasulfonate NF031 was also active in all four cell lines; in three cell lines it was significantly more active than suramin. Similarly, the tetrasulfonate NF326 was active in all cell lines, but less potent than suramin. In contrast the disulfonate NF062 showed a more selective activity. In T47D cells it was significantly more active than suramin (IC_{50} 24 versus 113 μM, respectively), while the antiproliferative activity in MCF7 cells and PAEC cells was comparable to that of suramin, and in PC3 cells no antiproliferative effect was observed. All other disulfonates were inactive.

In PC3 cells only one suramin analog, the hexasulfonate NF031, showed a higher antiproliferative activity than suramin (IC_{50} 55 and 98 μM, respectively).

With PAEC cells, similar antiproliferative activities were observed for suramin and the tetrasulfonate NF289. The hexasulfonate NF031 and the disulfonate NF062 demonstrated good antiproliferative activities with IC_{50} values of 100 and 74 μM, respectively, whereas all other disulfonates were inactive.

The asymmetric compound NF520, one half of the suramin molecule, was also inactive in all screened cell lines.

Table 2. Antiproliferative activity (IC_{50}[a]) of suramin analogs in PC3, MCF7, T47D and PAEC cells

Compound	PC3 cells	MCF7 cells	T47D cells	PAEC cells
Suramin	98	85	113	57
NF031	55	29	53	100
NF062	>500	100	24	74
NF108	>500	>500	>500	>500
NF109	>500	>500	>500	>500
NF241	>500	–	–	>500
NF289	–	–	–	40
NF326	190	110	120	169
NF499	–	>500	–	>500
NF500	>500	>500	>500	–
NF520	>500	>500	>500	>500

[a]Concentration (μM) inducing 50% growth inhibition.

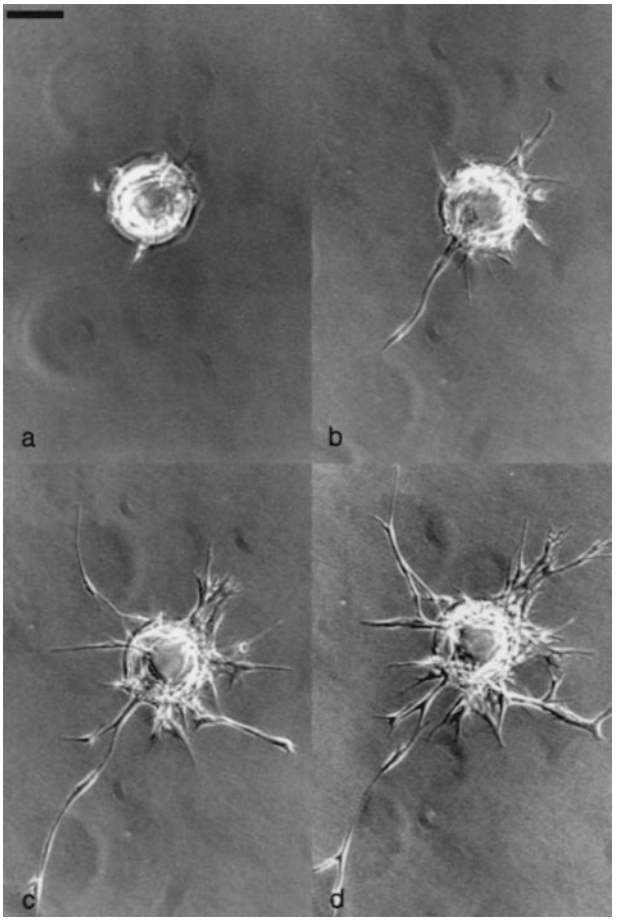


Figure 2. Vessel sprouting of untreated endothelial cells from Cytodex MCs in fibrin gels at day 1 to 4 (scale bar=120 μm).

Angiostatic activity

In the second step, the angiostatic activity of suramin analogs was determined by using the MC assay.

Cytodex MCs coated with porcine endothelial cells were embedded in fibrin. Starting at day 1 after polymerization of the gels, the cells formed capillaries in three-dimensional fibrin matrices. An example of the growth pattern from day 1 to 4 is given in Figure 2 (a–d).

Suramin and suramin analogs significantly inhibited the sprouting to a variable extent, inducing typical alterations. With suramin (100 μM), we initially observed vessel sprouting in a similar manner as in the control gels, but at day 3 and 4 cell sprouts demonstrated retraction and thinning (Figure 3a–d). Compared to the control gels, suramin-treated endothelial cells showed less vessel formation in the fibrin gels at day 4 (Figure 3a–d).

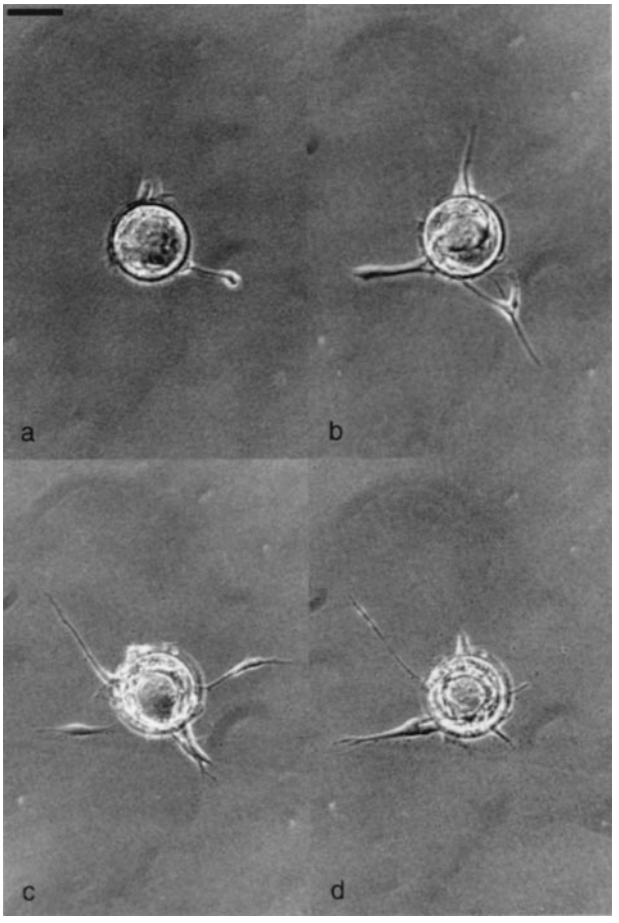


Figure 3. Decreased vessel sprouting of suramin-treated endothelial cells from Cytodex MCs in fibrin gels at day 1 to 4 demonstrating retraction and thinning of endothelial cells (scale bar=120 μm).

Suramin analogs were tested using concentrations ranging between 25 and 200 μM (Figure 4a and b). IC_{50} values of suramin analogs are given in Table 3. Suramin analogs inhibiting tumor cell proliferation also inhibited angiogenesis in the MC assay.

Suramin was the most potent angiostatic compound in this assay, inhibiting vessel sprouting slightly better than NF062, the only effective disulfonate.

The tetrasulfonates NF289 and NF326 also demonstrated angiostatic activities as well as the hexasulfonates NF031, NF037 and NF675.

The screened compounds significantly inhibited vessel sprouting with the exception of NF108 (Figure 4b).

An additional experiment with suramin and NF062 was performed demonstrating renewed proliferation of endothelial cells following compound removal at day 2 (data not given).

Discussion

The clinical use of suramin is limited by its significant toxicity. In previous studies, we and others have found that certain suramin analogs exhibit superior activities in different tumor cell lines and angiogenesis systems.[13,27,35] Interestingly, Braddock *et al.*[27] have observed that smaller suramin analogs with only four sulfonate groups retained significant angiostatic and antiproliferative activities, and were less toxic than suramin. They observed a 5- to 10-fold higher maximum tolerated dose in the mouse with certain smaller suramin analogs. Thus, the polyanionic structure may be an important contributor of toxicity. Therefore, we investigated suramin analogs with a reduced number of sulfonate groups and with varying molecular dimensions. In our study we observed that several of these analogs significantly inhibited tumor cell proliferation and angiogenesis using a recently developed MC assay.

Suramin and suramin analogs are known to be angiostatic[13,27,28,35] and tumors grow angiogenesis dependent.[29] Therefore, it seemed important to use a mammalian model for angiogenesis allowing optimum reproducibility and quantification. In the CAM model the vascularized areas are evaluated through a predefined score only with differentiation in good, slight and no inhibition of vessel growth only.[13,28] Another disadvantage is the high lethality of chicken embryos in this assay. Only a local effect of the tested compounds on the CAM is observed. Moreover, the extent of diffusion from the methylcellulose discs for different substances remains unknown. In addition, in mammalians results may be different from those in chicken. The rat or mouse sponge model, however, is technically demanding, expensive and also difficult to quantitate.[35]

The new *in vitro* angiogenesis assay developed by Nehls *et al.*[30,31] and used in this investigation is a combination of endothelial cell migration and capillary morphogenesis. Endothelial cells grow on small dextran beads until confluence and then form capillaries while growing in a three-dimensional fibrin gel. Reproducibility and easy quantification make this assay a valuable tool in angiogenesis research.

In our investigations, suramin was the best inhibitor of angiogenesis in this assay. However, several di- and tetrasulfonate analogs also showed significant activities.

Interestingly, we observed that the antiproliferative effect of suramin analogs on tumor cell lines paralleled to some extent their angiostatic activity. Thus, suramin analogs inhibiting tumor cell proliferation also demonstrated angiostatic activities. However, three suramin

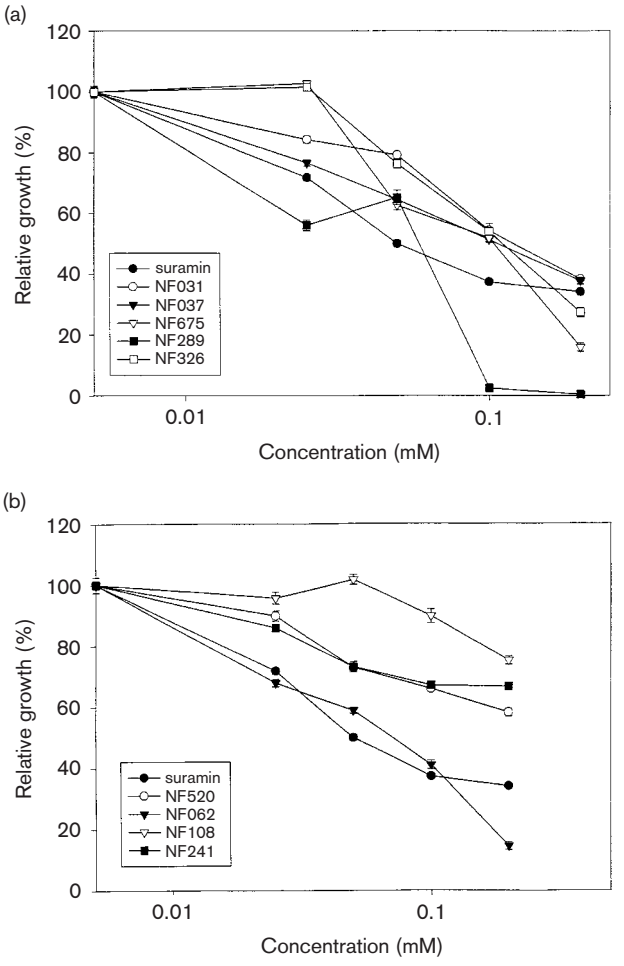


Figure 4. Angiostatic effect of suramin and suramin analogs. MCs coated with endothelial cells were embedded in fibrin gels and incubated in DMEM with 0.2% ITS in the presence of suramin or suramin analogs (25–200 μM final concentration) for 4 days. With each suramin analog, a minimum of three experiments was evaluated. Vessel growth curves were evaluated for determination of IC_{50} by the MC assay.

Table 3. Angiostatic activity (IC_{50}[a]) of suramin and suramin analogs in a new MC angiogenesis model

Hexasulfonates		Tetrasulfonates		Disulfonates	
Compound	IC_{50}	Compound	IC_{50}	Compound	IC_{50}
Symmetric suramin analogs					
Suramin	50	NF289	63	NF062	75
NF031	131	NF326	117	NF108	>500
NF037	115			NF241	>500
NF675	105				
Asymmetric suramin analogs					
NF520	IC_{50} >500				

[a]Concentration (μM) inducing 50% inhibition of angiogenesis.

analogs superior to suramin in inhibiting tumor cell proliferation were slightly less effective than suramin in the MC assay. Thus, most likely different mechanisms of action are involved in the activity of suramin and suramin analogs in different test systems. This view is supported by the observation that the disulfonate NF062 is an excellent inhibitor of angiogenesis and cell proliferation in HT29, MCF7 and PAEC cells, but not in PC3 cells. Similarly, the hexasulfonate NF031 demonstrated a lower activity in PAEC cells than in the tumor cell lines.

The number of sulfonate groups in the suramin analogs seems to be of minor importance because hexa-, tetra- and disulfonate analogs show similar antiproliferative and angiostatic activities. Of special interest is the disulfonate NF062. In NF062, one half of the naphthyl residues of suramin bearing two sulfonate residues is cut off. Therefore, the activities of NF062 indicate that the two sulfonate groups in positions 1 and 3 of the naphthyl residues are of minor importance for the angiostatic and antiproliferative activities of suramin, and that the sulfonate groups in the 5 position (in the *para* position of the amino group) of the naphthyl residues seem to be most important.

However, the distance between the opposite sulfonate groups in the symmetric molecules seems to be important too. In other words, a minimum molecular size is necessary for the angiostatic and antiproliferative activities of suramin analogs. The minimum molecular size should correspond to the 'small urea' type (e.g. NF326 and NF289). All molecules smaller than the 'small ureas' are inactive (e.g. NF108, NF109, NF499, NF500 and NF241).

Suramin is a nearly flat and, due to its amide bonds, rather rigid molecule. The preferred conformation of suramin will be a stretched form with the distance of the two trisulfonaphthyl residues reaching a maximum. The binding site of suramin might have two cationic regions at a distance corresponding approximately to the distance of the opposite anionic regions in the suramin molecule. This has been shown for four glycolytic enzymes isolated from the glycosomes of *Trypanosoma brucei* containing a marked excess of positive charges distributed in two or more clusters (called 'hot spots' by the authors) along the polypeptide chain.[36] Later, it was shown that these enzymes are selectively inhibited by suramin.[37]

Interestingly, the highly flexible suramin analogs NF528 and NF529 are inactive. These results support the hypothesis that a rigid structure is important for the biological activities of suramin analogs. The molecular dimensions of these hexasulfonates are very similar to those of suramin. Because of their flexibility,

a perfect fit to a cationic binding site should be possible. However, due to their flexibility these molecules could bind to a protein surface without influencing the conformation of the binding protein. On the other hand, the binding of a rigid molecule like suramin to a protein surface will certainly strongly influence the conformation of the protein, thus altering the biological function of the protein.

The central urea bridge is not essential for the antiproliferative and angiostatic activities of the suramin analogs. This is proven by the activities of the isophthalic acid diamide derivatives NF674 and NF675.

Our results are partly in agreement with recent results of Gagliardi *et al.*,[38] who found seven suramin analogs with better angiostatic activities than suramin using the CAM assay. The findings of Gagliardi *et al.*[38] suggest better angiostatic activities for hexasulfonate analogs of the 'large urea' type. The small symmetric disulfonate NF109, a benzenesulfonic acid derivative, was inactive in their investigation and in our test systems. However, in contrast to their results, the tetrasulfonate NF326 and the disulfonate NF062 showed good antiproliferative activities and angiogenesis inhibition in our test systems. Thus, the angiostatic activities of suramin analogs in the CAM assay may not be representative for other angiogenesis models and vice versa.

With the exception of NF520, all investigated suramin analogs are symmetric molecules. The non-symmetric compound NF520, one half of the suramin molecule, was not angiostatic in the microcarrier system. In a previous study[13] we have found that symmetry of the suramin analogs is essential for their antiproliferative activity. Similar observations were made for the inhibition of HIV reverse transcriptase of suramin analogs—asymmetric analogs were much less active than their corresponding symmetric analogs.[26] Therefore, we assume that a symmetric molecular structure of the suramin analogs is essential for their antiproliferative and angiostatic activities.

Interestingly, Morris *et al.*[39] have recently shown that the asymmetric suramin analog Eriochrome Black T (Erio T) is angiostatic in the CAM assay and also exhibits antiproliferative activity against tumor cells. Erio T is a derivative of 1-naphthylamine-4-sulfonic acid. However, its chemical structure differs significantly from the structures of suramin analogs described in this paper (e.g. it has chelating properties in contrast to our suramin analogs). Thus further investigations are warranted for a better understanding of the role of symmetry for antitumor activity of suramin analogs.

In conclusion, some suramin analogs with a reduced number of sulfonate groups retain significant antiproliferative and angiostatic activities. As previous studies[27] suggest a lower toxicity for such analogs they represent interesting compounds for further *in vivo* investigation in tumor models.

Furthermore, as the polyanionic character of suramin is probably responsible for its toxicity, the disulfonate NF062 may be a promising less toxic compound. However, the assumption of an improved activity/toxicity ratio for NF062 needs to be confirmed in *in vivo* studies.

Conclusion

Only suramin analogs which are characterized by a typical molecular structure show biological activity. A certain minimum molecule size, rigidity of the molecule and sulfonate groups in the *para* position seem to be important for angiostatic and antiproliferative activity.

The suramin analogs NF062, NF289 and NF326 may be considered as promising candidates for further studies.

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