Pergamon

Biochemical Pharmacology, Vol. 47, No. 2, pp. 295–302, 1994.

0006-2952/94 $6.00 + 0.00

NEW SULFONATED DISTAMYCIN A DERIVATIVES WITH bFGF COMPLEXING ACTIVITY

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(*Received* 15 *July* 1993; *accepted* 21 *September* 1993)

Abstract—Tumor-induced neoangiogenesis is an essential event for solid tumor growth. Therefore, a compound able to block angiogenesis-promoting factors could have antitumor activity. The polysulfonated naphthylurea suramin is hypothesized to have this mode of action. A series of sulfonated distamycin A derivatives have been synthesized with the objective of identifying novel compounds able to complex basic fibroblastic growth factor (bFGF) and other factors involved in tumour angiogenesis, and consequently to block the angiogenic process. These new compounds have been characterized for their ability to inhibit bFGF binding, *in vivo* bFGF-induced angiogenesis and neovascularization of the chorioallantoic membrane, in comparison with suramin. The two most active compounds, FCE 26644 [7,7′-(carbonyl-bis(imino-*N*-methyl-4,2-pyrrolecarbonyl-imino(*N*-methyl-4,2-pyrrole)carbonylimino))-bis(1,3-naphthalenedisulfonic acid)] and FCE 27164 [7,7′-(carbonyl-bis(imino-*N*-methyl-4,2-pyrrolecarbonyl-imino(*N*-methyl-4,2-pyrrole) carbonylimino)-bis (1,3,5-naphthalenetrisulfonic acid)] have been selected for extended evaluation. Both compounds are active in inhibiting platelet-derived growth factor β (PDGFβ) and interleukin-1β binding. Two different assays have been performed to study their mode of action: the sequential binding assay on bFGF and PDGF receptors and the bFGF-induced tyrosine phosphorylation assay. The results of the two assays are in agreement and indicate that no activity is observed if FCE 26644, FCE 27164 and suramin are administered as pretreatment, when a direct interaction of the compounds with bFGF and PDGF receptors is required. Conversely, inhibitory activity is observed when the compounds are allowed to form complexes with the growth factors themselves.

Angiogenesis, the growth of new blood vessels, is an essential component in development, progression and metastasis [1–3]. It has been suggested that tumors secrete angiogenic peptides which stimulate endothelial cells to proliferate and to form neovessels which provide the blood supply necessary for tumor growth [4, 5]. Therefore, a compound able to block angiogenesis-promoting factors could have antitumor activity.

The polysulfonated naphthylurea suramin is hypothesized to have this mode of action [6]. This compound is currently being investigated as an antitumor agent for the treatment of advanced malignancies and has exhibited antitumor activity in a number of tumors [7–9]. It exerts a wide variety of biological effects by inhibiting a large number of enzymes [10–12] and blocking the activity of several growth factors: EGF† [13], PDGFβ [14], interleukin-2 [15], TNFα [16], insulin-like growth factor 1 [17] and, in particular, bFGF [18], which is involved in tumoral angiogenesis [19–22].

With the objective of identifying novel compounds able to complex bFGF and consequently to block the angiogenic process, we have synthesized a series of sulfonated distamycin A derivatives. The new compounds have been characterized for their ability to inhibit bFGF binding, *in vivo* bFGF-induced angiogenesis and CAM neovascularization.

Here we report the results obtained in these assays with two families of closely related compounds: the members of the first family differ in the number of SO_3 groups, those of the second family differ in the skeleton. Moreover, we present an extended evaluation of the mode of action of two selected compounds, FCE 26644 [7,7′-(carbonyl-bis(imino-*N*-methyl-4,2-pyrrolecarbonyl-imino(*N*-methyl-4,2-pyrrole)carbonylimino)) - bis(1,3-naphthalenedisulfonic acid)] and FCE 27164 [7,7′-(carbonyl-bis-(imino-*N*-methyl-4,2-pyrrolecarbonyl-imino(*N*-methyl-4,2-pyrrole) carbonylimino) - bis(1,3,5-naphthalenetrisulfonic acid)]: activity on the binding of different growth factors and on bFGF-receptor associated tyrosine phosphorylation.

MATERIALS AND METHODS

Materials

bFGF, PDGFβ, EGF and IL-1β, both labeled and unlabeled, and ^{125}I-labeled whole antibody (from

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† Abbreviations: bFGF, basic fibroblast growth factor; PDGFβ, platelet-derived growth factor β; EGF, epidermal growth factor; IL-1β, interleukin-1β; TNFα, tumor necrosis factor α; BSA, bovine serum albumin; PBS, phosphate-buffered saline; CAM, chorioallantoic membrane; TBS, Tris-buffered saline; MEM, minimum essential medium.

sheep) antimouse immunoglobulin were purchased from Amersham (U.K.); HEPES, culture media and fetal calf serum from Gibco (Paisley, U.K.); bovine serum albumin, bacitracin and heparin from Sigma Chemical Co. (St Louis, MO, U.S.A.); suramin (Germanin) from Bayer (Leverkusen, Germany); antiphosphotyrosine PY20 antibody from ICN (U.K.) and gelatin sponges (gelfoam) from Upjohn (Kalamazoo, MI, U.S.A.). Distamycin A derivatives are original Farmitalia Carlo Erba compounds. Synthesis has been described elsewhere [23].

Culture conditions

For these studies, murine Balb/c 3T3 fibroblasts were used because they have bFGF, PDGFβ, EGF and IL-1β receptors. Balb/c 3T3 fibroblasts were grown in MEM with Earle's salts supplemented with 10% fetal calf serum and 1% non-essential aminoacids in a 5% CO_2 water-saturated atmosphere in 75-cm^2 plastic flasks. Cells were split twice a week to a density of 6000 cells/cm^2.

Binding assays

Balb/c 3T3 cells were seeded in 2-cm^2 wells of 24-multiwell plates at 5×10^3/cm^2. Four days after plating, confluent monolayers of Balb 3T3 cells were washed twice with binding medium: MEM + 0.2% BSA + 100 μg/mL bacitracin + 20 mM HEPES (+10 U/mL heparin for bFGF binding only).

Competition assay. The cells were incubated for 4 hr at 4° with scalar concentrations of tested compounds in a volume of 0.25 mL binding medium and a fixed concentration of ^{125}I-growth factors (0.2 nM bFGF, 0.2 nM EGF, 0.1 nM PDGFβ or 0.3 nM IL-1β).

Receptor assay. The sequential binding assay was performed essentially as described by Hosang [14]. The cells were incubated for 2 hr at 4° with 0.25 mL binding medium containing scalar concentrations of tested compounds. Then the cells were washed twice on ice with 1 mL cold (4°) binding medium and finally incubated for 4 hr at 4° with 0.25 mL binding medium containing a fixed concentration of ^{125}I-labeled growth factors (0.2 nM bFGF or 0.1 nM PDGFβ).

In both assays, at the end of the incubation, the cells were washed twice on ice with cold PBS and lysed with 0.5 mL hot (65°) 0.1 M NaOH, 2% Na_2CO_2, 2% sodium dodecyl sulfate (SDS) and transferred to tubes for counting in a γ-counter (Kobra autogamma Packard). Non-specifically bound radioactivity was determined in parallel cultures treated with an excess (1000 ×) of the corresponding unlabeled growth factors.

Antiphosphotyrosine blots

Balb/c 3T3 cells were seeded at 1.5×10^4/cm^2 in growth medium and were used for the assay 4 days after plating when confluent and growth-arrested. Cultures were washed twice with serum-free medium and exposed to the reported concentrations of the test compounds for 10 min at 37°. For sequential treatments, after the first treatment the cells were washed once with serum-free medium and exposed to the second treatment for 10 min. After exposure, the medium was quickly removed and the cells were solubilized in boiling Laemmli buffer. The cell lysates were immediately boiled for 5 min and sonicated. In each sample (diluted 1:10 or more) the protein content was estimated by the Pierce protein assay (bicinchoninic acid assay) and adjusted to an equivalent concentration in order to load 300 μg of protein/lane for separation by SDS–PAGE on a 10% acrylamide slab gel.

The SDS–PAGE separated proteins were transferred electrophoretically to a nitrocellulose sheet as described [24]. After transfer, the nitrocellulose sheets were soaked for 1 hr at 45° with a TBS–BSA 5% solution [150 mM TRIS–HCl, pH 7.4; 5% (w/v) BSA] and incubated overnight at 4° in the same TBS–BSA 5% buffer solution containing 2 μg/mL of monoclonal antiphosphotyrosine antibody PY20. Blots were repeatedly washed with TBS and thereafter incubated with ^{125}I-labeled anti-mouse immunoglobulin at room temperature for 2 hr.

Blots were dried, mounted and exposed with an intensifying screen for autoradiograms for 1 or 2 days. The molecular weight of the labeled phosphoproteins was estimated relative to the electrophoretic mobility of a cotransferred, prestained protein standard (Amersham, U.K.).

Chorioallantoic membrane (CAM) assay

The test was performed as described by Crum *et al.* [25]. Briefly, chicken embryos were removed from their shells on day 3 of development, placed in plastic Petri dishes (Corning, NY, U.S.A.) and maintained at 37°, 3% CO_2. On day 5 embryos were treated with the test molecules incorporated into a 0.5% (w/v) methylcellulose disk placed on top of growing CAMs. Controls were treated with saline. A group of 10 eggs was used for each dose.

After 48 hr, embryos were observed with a stereomicroscope (Carl Zeiss, Germany) at 10× to assess the density of capillaries around the disks. Embryos presenting avascular zones at least 2 mm in diameter were considered positive.

The results are reported as % of avascular CAM (positives) at the tested concentration.

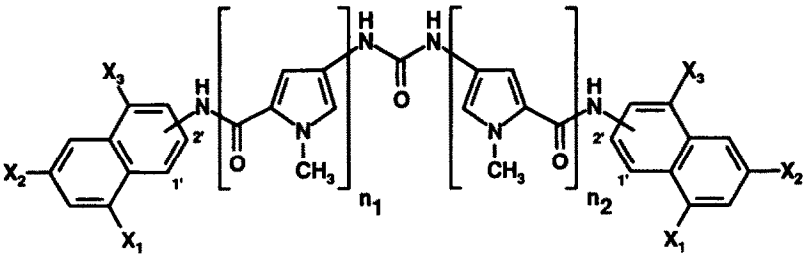
An inhibition >50% was considered significant with $P < 0.01$ using the Student's *t*-test.

bFGF angiogenesis assay

Gelatin sponges cut into strips (approximately 7 by 10 mm) under sterile conditions were loaded with 0.25 mL of a PBS–BSA 0.1% solution supplemented with 30 μg/mL bFGF. Control sponges were prepared in the same way and impregnated with PBS–BSA 0.1%.

C3H/He, adult female mice were used to evaluate the antiangiogenic activity on the sponge model. All animals were supplied by Charles River (Calco, Como, Italy). The conventional mice were 2–3 months old, weighed 20–22 g and were kept under standard laboratory conditions. The mouse colony was routinely tested for the absence of antibodies to a panel of pathogens including: mouse hepatitis, Sendai virus and *Mycoplasma pulmonis*.

Following induction of anesthesia, a 1-cm long dorsal middle skin incision was made proximately to the base of the tail and by gentle dissection with forceps a subcutaneous pouch fashioned 2–3 cm



FCE 26605 :	$X_1=X_2=X_3=SO_3Na$		1' N	$n_1=n_2=2$
FCE 27164 :	$X_1=X_2=X_3=SO_3Na$		2' N	$n_1=n_2=2$
FCE 27192 :	$X_1=X_2=SO_3Na$	$X_3=H$	1' N	$n_1=n_2=2$
FCE 26644 :	$X_1=X_2=SO_3Na$	$X_3=H$	2' N	$n_1=n_2=2$
FCE 26580 :	$X_2=SO_3Na$	$X_1=X_3=H$	1' N	$n_1=n_2=2$
FCE 26951 :	$X_1=X_2=SO_3Na$	$X_3=H$	2' N	$n_1=n_2=1$
FCE 27111 :	$X_1=X_2=SO_3Na$	$X_3=H$	2' N	$n_1=n_2=3$
FCE 27163 :	$X_1=X_2=SO_3Na$	$X_3=H$	2' N	$n_1=3\ n_2=1$

Fig. 1. Chemical structure of the newly synthesized distamycin A derivatives.

cephalad to the incision. After implantation of the sponge into the s.c. pouch, the skin was sutured. All drug solutions were prepared immediately before use.

Treatment was administered i.v. at a dose of 200 mg/kg on day 1. Fifteen days after he implantation, mice were killed and sponges were surgically extracted and prepared for histological examination.

The % inhibition of vascularization was evaluated on the extracted sponge by counting the vessels, and the following formula was used:

$$100 - \frac{\text{N treated sponge}}{\text{N positive control sponge}} \times 100$$

where N is the mean number of vessels counted in the sponge. An inhibition >50% was considered significant with $P < 0.01$ using the Student's *t*-test.

RESULTS

Chemical structure

The general chemical structure of the newly synthesized distamycin A derivatives is presented in Fig. 1. All compounds possess a central moiety formed by a repeated module of *N*-methylpyrrole-*r*-carboxamide that connects two terminal units of *α*- or *β*-naphthylamine differently substituted by sulfonic groups.

Structure–activity relationship studies

The new compounds have been tested in three different assays. The binding assay measures their ability to inhibit bFGF binding to its receptor, the inhibitory activity is expressed as ID_{50}. The bFGF angiogenesis assay determines the *in vivo* inhibitory activity on experimental bFGF-induced angiogenesis; the activity is expressed as percentage inhibition. The CAM assay determines the effect on physiological neovascularization of the chicken embryo CAM; the inhibitory activity is expressed as percentage of CAMs with an avascular area.

Table 1 reports the results obtained with a class of compounds that differ in the number of SO_3 groups, both for *α*- and *β*-naphthylamine derivatives, in comparison with suramin. FCE 26644 is the most active compound, presenting an ID_{50} value of $142 \pm 18\ \mu M$ in the binding assay and an inhibition value of 100% both in the bFGF angiogenesis assay and in the CAM assay. FCE 27164 is also very active in all assays: ID_{50} $116 \pm 10\ \mu M$ in the binding assay and 93% and 80% inhibition in the bFGF angiogenesis assay and the CAM assay, respectively. The results obtained with suramin are comparable to those reported in the literature by our group [6, 26] or other groups [13, 18]. Table 2 shows the results obtained in the same assays with compounds that share the naphthyl moiety of FCE 26644, the most active compound presented in Table 1, and

Table 1. Screening of distamycin derivatives: compounds that differ in number of SO_3 groups

Compound	Inhibitory activity on: bFGF binding ID_{50}* (μM)	bFGF-induced angiogenesis inhibition† (%)	CAM vascularization inhibition‡ (%)
Suramin	91 ± 11	94	87
FCE 26605	153 ± 15	80	60
FCE 27164	116 ± 10	93	80
FCE 27192	390 ± 34	82	77
FCE 26644	142 ± 18	100	100
FCE 26580	857 ± 109	0	45

* Dose inhibiting growth factor binding by 50% in a contemporaneous assay. Three different experiments are carried out in duplicate.

† Evaluated as:

$$100 - \frac{\text{N treated sponges}}{\text{N positive control sponges}} \times 100$$

where N is the mean number of vessels counted in 10 fields of histological sections. Treatment dose: 200 mg/kg i.v. at day 1. At least two different experiments were carried out with five mice per treatment.

‡ Evaluated as:

$$\frac{\text{No. of CAMs with inhibited area}}{\text{No. of CAMs treated}} \times 100.$$

Treatment dose: 350 nm/pellet. At least two different experiments were carried out with 10 embryos per treatment.

Table 2. Screening of distamycin derivatives: compounds that differ in the central moiety

Compound	Inhibitory activity on: bFGF binding ID_{50}* (μM)	bFGF-induced angiogenesis inhibition† (%)	CAM vascularization inhibition‡ (%)
FCE 26644	142 ± 18	100	100
FCE 26951	550 ± 92	ND	30
FCE 27111	163 ± 16	89	62
FCE 27163	252 ± 132	100	83

* Dose inhibiting growth factor binding by 50% in a contemporaneous assay. Three different experiments are carried out in duplicate.

† Evaluated as:

$$100 - \frac{\text{N treated sponges}}{\text{N positive control sponges}} \times 100$$

where N is the mean number of vessels counted in 10 fields of histological sections. Treatment dose: 200 mg/kg i.v. at day 1. At least two different experiments were carried out with five mice per treatment.

‡ Evaluated as:

$$\frac{\text{No. of CAMs with inhibited area}}{\text{No. of CAMs treated}} \times 100.$$

Treatment dose: 350 nm/pellet. At least two different experiments were carried out with 10 embryos per treatment.

differ in the number of methylpyrrole rings and in their position relative to the ureidic carbonyl. A significant decrease in the binding inhibitory activity is observed with the shortest molecule (FCE 26951), whereas no increase in activity is observed with the longest spanning compound (FCE 27111).

In the bFGF–gelfoam and CAM assays, good activity is observed with the asymmetric compound FCE 27163 (100% and 83% inhibition) and with the longest compound (89% and 62% inhibition), but no FCE 26644 activity is observed. Based on these results, FCE 26644 and FCE 27164 have been selected for an extended evaluation of the mode of action.

Table 3. Effect of FCE 26644 and suramin on growth factor binding in a competition assay

Compound	ID_{50}* (μM) for binding of: bFGF	PDGFβ	EGF	IL-1β
Suramin	91 ± 11	31 ± 0	716 ± 113	140 ± 20
FCE 26644	142 ± 18	55 ± 3	471†	450 ± 125
FCE 27164	116 ± 10	17 ± 1	NT	313 ± 8

Competition assay: cells are exposed to the compound in the presence of labeled growth factors for 4 hr at 4°.

* Dose inhibiting growth factor binding by 50%. The results are representative of at least three experiments carried out in duplicate with the exception of †.

NT, not tested.

Binding competitition assay

Table 3 reports the results obtained testing the effect of FCE 26644, FCE 27164 and suramin treatment on the binding of PDGFβ and EGF, which have been reported to participate in tumoral angiogenesis [27, 28], and IL-1β, which has been suggested to play an important role in the implant of metastatis [29], to their receptors on Balb/c 3T3 murine fibroblasts in competition assays. FCE 26644 and suramin present comparable ID_{50} values on the binding of PDGFβ and EGF. Both compounds are most efficient in inhibiting PDGFβ binding and present reduced activity on EGF binding.

FCE 27164 is the most active in inhibiting PDGFβ binding ($ID_{50} = 17 \pm 1\ \mu M$). Both FCE 26644 and FCE 27164 are less effective than suramin in inhibiting IL-1β binding with ID_{50} values of 450 ± 125 and $313 \pm 8\ \mu M$, respectively, in comparison with $140 \pm 20\ \mu M$ for suramin. The IL-1β binding inhibitory activity of suramin has not been previously reported in the literature; for EGF and PDGFβ suramin ID_{50} values are comparable to those reported in the literature [13, 14].

Receptor binding sequential assay

The sequential binding assay makes it possible to distinguish whether binding inhibitory activity is due to the formation of a complex between the compounds and the growth factors or the corresponding receptors. This assay has already been described in the study of the interaction of suramin with PDGFβ [14]. Table 4 reports results obtained testing the effect of FCE 26644, FCE 27164 and suramin pretreatment on the binding of bFGF and PDGFβ to their receptors. In this assay, all compounds are unable to prevent the binding of bFGF and PDGFβ to their receptors with ID_{50} values $>1000\ \mu M$. Conversely, pretreatment with unlabeled bFGF and PDGFβ inhibits the binding of the corresponding labeled growth factor with ID_{50} values of 0.074 ± 0.020 and $0.129 \pm 0.011\ \mu M$, respectively. These data indicate that the inhibition of bFGF and PDGFβ binding is not due to a direct interaction of the compounds with the receptors, as already reported for PDGFβ and suramin [14].

Tyrosine phosphorylation assay

The assay determines the effect of the compounds on the physiological activation of the bFGF receptor on Balb/c 3T3 cells. These cells have the advantage of being easily activated upon treatment with exogenous bFGF. In fact, as shown in Fig. 2, lane 2, treatment with bFGF (10 ng/mL) increases the tyrosine phosphorylation of a protein with a molecular weight of 92 kDa. A p90 has already been reported as the major substrate of specific phosphorylation which is strongly correlated with the proliferative effects after bFGF stimulus [30].

Table 4. Effect of FCE 26644 and suramin pretreatment on bFGF and PDGFβ binding to their receptors

	ID_{50}* (μM) for binding of: bFGF	PDGFβ
Unlabeled PDGFβ	—	0.129 ± 0.011
Unlabeled bFGF	0.074 ± 0.020	—
Suramin	>1000	>1000
FCE 26644	>1000	>1000
FCE 27164	>1000	>1000

Sequential assay: cells are exposed for 2 hr at 4° to the tested compounds, washed twice and treated for 4 hr at 4° with the labeled growth factors.

* Dose inhibiting growth factor binding by 50%. The results are representative of two experiments carried out in triplicate.

The p92 phosphorylation is more relevant than the autophosphorylation of the bFGF receptor (p145) and particularly more stable; in fact, it is maintained up to 30 min after the cells have been washed [26]. In this way sequential treatments can be performed, also allowing the study of the activity of the compounds when the binding bFGF–bFGF receptor has already taken place.

Figure 2 presents the results: whereas treatment with FCE 26644 and suramin (1 mM) alone does not have any effect (lanes 3 and 4, respectively), inhibition of bFGF-induced p92 tyrosine phosphorylation is observed both when cells are treated contemporaneously with 10 ng/mL bFGF and 1 mM FCE 26644 (lane 8) or suramin (lane 11), and when cells are first stimulated with bFGF, washed and treated with one of the compounds (FCE 26644 in

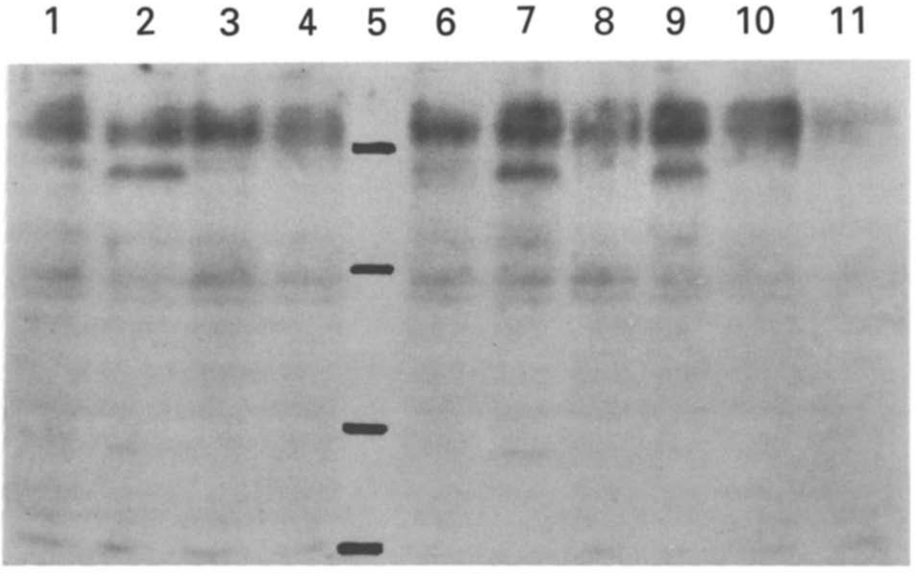


Fig. 2. Effect of FCE 26644 and suramin on bFGF-induced tyrosine phosphorylation. Cell treatments are performed in serum-free medium with a fixed concentration of the compounds (10 ng/mL bFGF, 1 mM suramin, 1 mM FCE 26644) for 10 min. Total proteins, extracted in Laemmli sample buffer, are separated by SDS–PAGE and transferred to a nitrocellulose sheet. The blot is probed with a monoclonal antiphosphotyrosine antibody, followed by ^{125}I-antimouse immunoglobulin. Lane 1: control; 2, bFGF; 3. FCE 26644; 4: suramin; 5: standard proteins (phosphorylase B 97.4 (molecular weight in kDa); bovine serum albumin 69; ovalbumin 46; carbonic anhydrase 30); 6: bFGF + wash + FCE 26644; 7: FCE 26644 + wash + bFGF; 8: bFGF + FCE 26644; 9: suramin + wash + bFGF; 10: bFGF + wash + suramin; 11: bFGF + suramin. This blot is representative of three experiments.

lane 6 and suramin in lane 10). Conversely, when cells are first treated with FCE 26644 (lane 7) or suramin (lane 9), washed and stimulated with bFGF, no inhibition is observed. Similar results have been obtained with FCE 27164 (not shown).

DISCUSSION

The aim of the present study was to identify new polysulfonated molecules able to block bFGF activities and in particular bFGF-induced neoangiogenesis which is an essential event for solid tumor growth. This is the mode of action hypothesized for a well-known polysulfonated compound, suramin. This old compound, widely used in the treatment and prophylaxis of trypanosomiasis and onchocerciasis [31], has recently exhibited antitumor activity in a number of systems. The main obstacle in the use of suramin in patients is its toxicity for adrenals and kidneys as well as its neurological side-effects [32–34]. Hence, the necessity of identifying compounds with the same mode of action as suramin but possessing reduced toxicity. The screening system utilized for the evaluation of new compounds was set up with the objective of identifying molecules able to inhibit angiogenesis by forming a complex with bFGF.

The two selected compounds showed considerable inhibitory activity in the bFGF binding assay and the ability to block bFGF-induced angiogenesis (100% and 93% inhibition with FCE 26644 and FCE 27164, respectively) and physiological neovascularization in the CAM (100% and 80% inhibition with FCE 26644 and FCE 27164, respectively).

Further studies have been performed to investigate the effect of FCE 26644 and FCE 27164 on the binding of several growth factors. The binding activity of FCE compounds did not differ from that of suramin with the exception of a lower inhibition of IL-1β binding. The inhibitory activity of suramin on IL-1β binding had not been described previously in the literature. IL-1β has been suggested to play an important role in the implant of metastasis [29].

Two different assays have been performed to study the mode of action of FCE compounds: the sequential binding assay, where only the molecules able to bind directly to the receptor are active, and the p92 tyr-phosphorylation assay. The results of the two assays were in agreement: in fact, pretreatment with FCE 26644, FCE 27164 and suramin did not affect either the binding of bFGF and PDGFβ to their receptors or bFGF-induced p92 tyr-phosphorylation. Conversely, inhibition of p92 tyrosine phosphorylation was observed not only when cells were treated contemporaneously with bFGF and the tested compounds but also when cells were first stimulated with bFGF, washed and treated with the compounds. This observation suggests that suramin and the FCE compounds are not only able to prevent the interaction between bFGF and its receptor but also can dissociate the bFGF already bound, blocking in this way all the biological responses.

In conclusion, the mode of action of FCE 26644 and FCE 27164, polysufonated compounds with antiangiogenic activity, is similar to that of suramin: the inhibition of bFGF activities is due to complex formation with the growth factor itself and not to direct interaction with the bFGF receptor. The complex bFGF–compound can form both when bFGF is free in the medium and when it is already bound to the receptor.

The antitumor activity of FCE 26644 and FCE 27164 in comparison to suramin has been evaluated against M5076 reticulosarcoma and found to be considerable (tumor growth inhibition 92% and 88%, respectively). In particular, FCE 26644 was active in inhibiting tumor growth with remarkable efficacy on M5076 reticulosarcoma, S180 sarcoma and MXT fibrosarcoma, all bFGF-producing tumors (manuscript in preparation). In these models, the antitumor activity of suramin was lower than or comparable to that of the FCE compounds. In all the tested models, FCE 2664 was remarkably less toxic than suramin.

Acknowledgements—This work was supported by I.M.I. on behalf of the Italian Ministry for Universities and Scientific and Technological Research.

We thank Mrs G. Viscardi for secretarial assistance.

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