Specific inhibition of the activity of the urokinase receptor-mediated cell-surface plasminogen activation system by suramin

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Urokinase-type plasminogen activator (uPA) is involved in generating the proteolytic activity necessary for invasive processes, and is dependent on binding to its specific cellular receptor (uPAR) for efficient function. We report here that the polysulphonated naphthylurea compound suramin inhibits the activity of this cell-associated proteolytic system, in a manner independent of its antagonism of the uPA–uPAR interaction [Behrendt, Rønne and Danø (1993) *J. Biol. Chem.* **268**, 5985–5989], occurring at a 25–100-fold-lower suramin concentration. This inhibition was found to be due to effects on the activation of both pro-uPA and plasminogen. Suramin inhibited plasmin activation of pro-uPA by a non-competitive mechanism (K_i approx. 2 µg/ml), which did not involve a direct effect on plasmin catalytic activity. Similarly, its effect on plasminogen

activation was not due to a direct inhibition of uPA. The inhibition of plasminogen activation, which occurred exclusively with receptor-bound uPA, appeared to be due to a reversal of the favourable kinetics which result from the activation of cell-associated plasminogen, although suramin did not inhibit the cellular binding of ^{125} I-labelled plasminogen. This suggests that this effect is due to interference with interactions between components of this system on the cell surface, and that suramin may be useful in gaining further insight into the molecular mechanisms involved in the functional assembly of this proteolytic system. Furthermore the effective inhibition of this system by suramin indicates an anti-invasive potential that may contribute to the anti-tumour effect of suramin *in vivo*.

INTRODUCTION

The polysulphonated naphthylurea, suramin, extensively used in the treatment of trypanosomiasis and onchocerciasis has recently been found to be of potential use in the treatment of certain metastatic cancers [1,2]. The mechanisms responsible for the effectiveness of suramin as an anti-tumour drug are poorly understood. They are thought to include both inhibitory effects on enzyme systems, e.g. DNA polymerases [3] and kinases involved in phosphoinositide metabolism [4], and the antagonism of the binding of several growth factors to their complementary cellular receptors [5–7]. In addition to such anti-proliferative and cytotoxic effects, it has also been suggested that suramin exerts anti-invasive effects via the inhibition of heparanase, an endo- β -D-glucuronidase implicated in basement-membrane degradation during tumour metastasis [8].

The invasive and metastatic properties of tumour cells are closely correlated to their potential to degrade components of the extracellular matrix [9]. This potential is largely mediated through proteolytic enzymes, including matrix metalloproteinases, certain cathepsins and plasminogen activators. Among these proteases urokinase-type plasminogen activator (uPA) has been postulated to play a central role [10]. Through the generation of the broad-specificity protease plasmin, it can mediate the degradation of most matrix proteins and activate certain pro-collagenases. There is compelling evidence that the activity of the uPA system of plasminogen activation *in vivo* is dependent on the binding of uPA to its specific cellular receptor, uPAR [11]. This comes from both enzyme kinetic studies [12,13] and the histological observation of widespread co-expression of uPA and uPAR in invasive areas of human tumours [11,14]. Some studies using model systems of cellular invasion also support the involvement of uPAR in these processes [15–17].

The dependence of the activity of this system on uPAR is not solely due to the high-affinity binding of uPA to uPAR, which is mediated through an epidermal growth factor-like domain in the proteinase [18,19], but requires in addition the concomitant cellular binding of plasminogen. Accordingly upon binding of the zymogen forms pro-uPA and plasminogen to U937 monocytoid cells, there is a large potentiation of plasmin generation [12], due to increased efficiencies of both the activation of plasminogen and the feedback activation of pro-uPA by generated plasmin [12,13]. It has recently been proposed that these kinetic effects are due to the formation of specific complexes on the cell surface, which may also be involved in the initiation of this reciprocal zymogen activation system [20].

Recently suramin has been demonstrated to antagonize the binding of uPA to uPAR [21], in a manner consistent with its effect on the interactions between growth factors and their receptors, as mentioned above. We report here that, independently of this effect, suramin is able to directly inhibit the activity of the uPAR-mediated plasminogen activation system. This involves a dual effect on plasmin-catalysed pro-uPA activation and the activation of cell-associated, but not solution-phase, plasminogen.

EXPERIMENTAL

Proteins and reagents

Human uPA, pro-uPA, [Glu¹]plasminogen, plasmin and the monoclonal antibody anti-uPA clone 1 were all as described previously [12,13,20]. Human plasma kallikrein and human neutrophil cathepsin G were obtained from Calbiochem (San Diego, CA, U.S.A.). The plasmin- and uPA-specific fluorogenic substrate H-D-Val-Leu-Lys-AMC and Z-Gly-Gly-Arg-AMC (where AMC is 7-amido-4-methylcoumarin and Z is a benzyloxy-

Abbreviations used: uPA, urokinase-type plasminogen activator (both the activated two-chain protease and as a generic term); pro-uPA, single-chain form of uPA; uPAR, uPA receptor; AMC, 7-amido-4-methylcoumarin; Z, benzyloxycarbonyl.

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carbonyl group) were from Bachem AG (Bubendorf, Switzerland). Suramin was obtained as Germanin from Bayer (Leverkusen, Germany). Plasminogen was labelled with Na¹²⁵I as described previously [13].

Measurements of cell-associated plasminogen activation

Plasmin generation by uPA or pro-uPA bound to cellular uPAR was determined essentially as described previously [12,13]. Briefly U937 cells were washed three times in RPMI 1640 buffered by 25 mM Hepes, pH 7.4, and were incubated for 3 min with 0.05 M glycine/HCl, pH 3.0, containing 0.1 M NaCl (to dissociate endogenously bound uPA [22]), followed by neutralization and further washing. The cells were resuspended at a density of 1×10^7 cells/ml and incubated with uPA or pro-uPA (2 nM) for 20 min at 37 °C, followed by three further washes. The cells were then incubated (37 °C) at a final density of 1×10^6 cells/ml in 0.05 M Tris/HCl, pH 7.4/0.1 M NaCl/0.01 % Tween 80 in the presence of 0.2 mM H-D-Val-Leu-Lys-AMC and [Glu¹]plasminogen at a fixed concentration of 50 nM or, in experiments in which kinetic constants were determined, with various concentrations up to 5 μ M. Suramin was included in these incubations at various concentrations as described in the appropriate figure legends. In some experiments 6-aminohexanoic acid was included in the incubation buffer at a concentration of 1 mM as an antagonist of the cellular binding of plasminogen [23]. Plasmin generation was determined by recording the level of released AMC fluorescence at 1-min-long intervals, using the conditions described previously [13]. The plasminogen activator activity observed under these conditions was demonstrated to be due solely to uPAR-bound enzyme by the criteria described previously [24]. It was also shown in control experiments using ¹²⁵I-labelled di-isopropylfluorophosphate-inactivated uPA that suramin caused no detectable (i.e. less than 5 %) dissociation of bound ligand over the 30 min time course of the experiments, nor internalization of bound ligand as determined by its quantitative release at pH 3.0.

Measurement of solution-phase plasminogen and pro-uPA activation

The activation of plasminogen by solution-phase uPA was determined by incubating uPA (0.5 nM) with [Glu¹]plasminogen (85 nM) in 0.05 M Tris/HCl, pH 7.4/0.1 M NaCl/0.01 % Tween 80/0.2 mM H-D-Val-Leu-Lys-AMC at 37 °C. Plasmin generation was then determined continuously as described above.

Plasmin-catalysed pro-uPA activation was determined by incubation of various concentrations of pro-uPA (2.9–11.4 nM) with plasmin (0.1 nM) in the presence or absence of various concentrations of suramin in 0.05 M Tris/HCl, pH 7.4/0.1 M NaCl/0.01 % Tween 80 containing 0.5 mM Z-Gly-Gly-Arg-AMC at 37 °C. Fluorogenic substrate hydrolysis was measured as above, and the rates of pro-uPA activation expressed as dF/dt . The effect of suramin on pro-uPA activation by kallikrein (10 nM) and cathepsin G (20 nM) was performed in essentially the same manner at a pro-uPA concentration of 50 nM and suramin concentrations up to 25 μ g/ml.

Suramin at concentrations up to 25 μ g/ml had no effect on the hydrolysis of either H-D-Val-Leu-Lys-AMC or Z-Gly-Gly-Arg-AMC by plasmin and uPA respectively. At higher suramin concentration (100 μ g/ml and above) non-specific inhibitory effects were observed with uPA, due to limited solubility of the peptide substrate Z-Gly-Gly-Arg-AMC in the presence of the polyanionic suramin.

Theoretical analysis of plasmin generation curves in the presence of anti-u-PA clone 1

Plasmin generation in the presence of the monoclonal antibody anti-uPA clone 1 was determined using methodology similar to that described above, and as previously described [20]. The effect of suramin on the kinetics of the two reactions potentiated by this antibody, i.e. both plasminogen activation and pro-uPA activation, were calculated using a previously described theoretical model [20]. In the absence of suramin the final rates of plasmin generation were fitted according to the previously reported kinetic constants, the small effect of suramin on the final plasmin generation rate was then calculated as a change in k_{cat}/K_m value. The effect of suramin on the kinetics of plasmin-catalysed pro-uPA activation was then determined by obtaining the best fit of the associated k_{cat}/K_m values to the experimental plasmin generation curves.

Binding assay for ¹²⁵I-labelled plasminogen

U937 cells were prepared as described above, and resuspended at a final density of 1×10^7 cells/ml in 0.05 M Tris/HCl, pH 7.4/0.1 M NaCl/0.1 % BSA containing 10 μ g/ml aprotinin. The cells were then incubated for 2 h at 4 °C with ¹²⁵I-labelled plasminogen (0.1 mBq/ml, approx. equivalent to 0.6 nM) and various concentrations of unlabelled plasminogen up to 10 μ M. Aliquots (100 μ l) of cell suspension were then layered on to 200 μ l of oil mixture (85:15, v/v of dimethyldiphenylpolysiloxane and $\rho = 0.88$ g/ml mineral oil) in polypropylene microcentrifuge tubes, before centrifugation at 14000 *g* for 3 min, amputation of the tube tips and γ -radioactivity counting. The effect of suramin on the binding of plasminogen was determined in two ways: first, specific plasminogen binding isotherms were determined in the presence and absence of 25 μ g/ml suramin; and secondly, the binding of ¹²⁵I-labelled plasminogen was measured at various concentrations of suramin up to 200 μ g/ml. Non-specific binding was defined as that not inhibited by 20 mM 6-aminohexanoic acid [25].

RESULTS

Suramin inhibits uPAR-mediated plasmin generation independently of its effect on uPA binding

It has recently been demonstrated that suramin antagonizes the binding of uPA to its cellular receptor. In studying the consequence of this effect for the activity of the cell-surface plasminogen activation system mediated by pro-uPA binding to uPAR on U937 cells, we observed that concentrations of suramin (1–10 μ g/ml) below those needed to antagonize receptor binding (IC_{50} 50–200 μ g/ml) had a large effect on the generation of plasmin (results not shown). This suggested that additional effects might be involved, possibly inhibitory effects on the proteolytic reactions, and therefore these experiments were repeated with pro-uPA pre-bound to the cells before addition of suramin. The result of such an experiment is shown in Figure 1 and it can be seen that under these conditions, where the effects on pro-uPA binding are eliminated, a large inhibition of plasmin generation is observed at a suramin concentration of 1 μ g/ml, and an almost complete inhibition at 25 μ g/ml.

In control experiments it was observed that pre-incubation of cells with suramin and washing before addition of pro-uPA had no effect on the resulting activity of the system. In contrast, incubation of cells having pre-bound pro-uPA with suramin and subsequent washing did have some inhibitory effect (approx. 50 % of the inhibitory effect at an original suramin concentration

Figure 1 Effect of suramin on the potentiation of pro-uPA-mediated plasmin generation on U937 cells

Pro-uPA was pre-incubated with U937 cells, which were then washed three times before addition of plasminogen (50 nM) and H-o-Val-Leu-Lys-AMC. Plasmin generation was measured continuously as described in the Experimental section. Suramin was included in the incubations at concentrations of 0 (○), 1 (▼), 10 (▲) and 25 $\mu\text{g/ml}$ (■).

Figure 2 Inhibition of plasmin-catalysed pro-uPA activation by suramin

Pro-uPA was incubated with various concentrations of suramin (up to 20 $\mu\text{g/ml}$) and the generation of uPA measured continuously by hydrolysis of Z-Gly-Gly-Arg-AMC after the addition of plasmin (0.1 nM). The results shown are plotted according to the method of Dixon [33], i.e. reciprocal of reaction rate (expressed in arbitrary fluorescence units) versus suramin concentration, at various concentrations of pro-uPA (▼, 2.9; ■, 5.7; ▲, 11.4 nM). The lines intercept on the x-axis indicating a non-competitive inhibitory mechanism, with a K_i value of 1.8 $\mu\text{g/ml}$.

of 100 $\mu\text{g/ml}$), suggesting that the binding of suramin to receptor-bound pro-uPA is at least partly responsible for the observed inhibition of plasmin generation.

Effect of suramin on uPA-catalysed plasminogen activation

To determine the cause of the suramin inhibition of receptor-bound pro-uPA-mediated plasmin generation, its effect on the two primary proteolytic reactions in this system, namely plas-

minogen activation by active, two-chain uPA and pro-uPA activation by plasmin, were studied using solution-phase reactants.

Suramin was found to affect plasminogen activation; however, this was a potentiatory effect rather than an inhibitory one, and as the effect was relatively small (a maximum 5-fold increase in plasmin generation at 25 $\mu\text{g/ml}$, results not shown) its kinetic mechanism was not studied further.

Effect of suramin on plasmin-catalysed pro-uPA activation

In contrast to its minor potentiatory effect on plasminogen activation, suramin was found to strongly inhibit plasmin-catalysed pro-uPA activation. Experiments were performed using a range of pro-uPA and suramin concentrations, and the results are shown in Figure 2. This Dixon plot reveals that suramin behaves as a non-competitive inhibitor of pro-uPA activation i.e. affecting only V_{max} and not K_m for the reaction, with the K_i calculated to be 1.8 $\mu\text{g/ml}$ (1.3 μM). This inhibitory mechanism is consistent with the observation that suramin is without effect on the activity of plasmin against low- M_r peptide substrates (see the Experimental section), and therefore is not a direct active-site inhibitor of plasmin.

The specificity of this inhibition was investigated using two other serine proteases reported to activate pro-uPA; plasma kallikrein [26] and cathepsin G [27]. Suramin up to a concentration of 25 $\mu\text{g/ml}$ had no effect on pro-uPA activation by either of these proteases.

Effect of suramin on the potentiation of plasmin generation mediated by an anti-uPA monoclonal antibody

To determine whether the non-competitive inhibition of plasmin-catalysed pro-uPA activation was sufficient to account for the overall inhibition of plasmin generation observed with receptor-bound pro-uPA, we made use of a putative model system for receptor-mediated plasminogen activation that we have recently described [20]. This is based on an anti-uPA monoclonal antibody that kinetically mimics the potentiatory effects of cell-associated uPAR on plasmin generation through ternary complex formation, simultaneously binding pro-uPA and plasminogen, the latter to a lysine residue at the C-terminus of the immunoglobulin heavy chain. As shown in Figure 3 suramin inhibits the overall generation of plasmin in the presence of this antibody, but the characteristics of this are not the same as those seen with U937 cells in Figure 1. In the presence of the monoclonal antibody the primary effect is an increase in the lag phase before the onset of rapid plasmin generation, suggesting an inhibition of pro-uPA activation, but with the final rate of plasmin generation increased. Fitting of these experimental data to a kinetic model using our previously published constants quantitatively demonstrates the validity of these qualitative assessments, with the catalytic efficiency of pro-uPA activation decreased 27-fold and plasminogen activation increased 2-fold at 25 $\mu\text{g/ml}$ (see legend to Figure 3), both being consistent with the effects of suramin on the individual reactions observed above. Therefore it appears that the inhibition of pro-uPA activation alone is not enough to account for the potent effect of suramin on the activity of the uPAR-mediated cell-surface plasminogen activation system.

Effect of suramin on the binding and activation of cell-surface-associated plasminogen

As the inhibition of this system by suramin appears to involve effects beyond the non-competitive inhibition of pro-uPA ac-

Figure 3 Effect of suramin on the potentiation of pro-uPA-mediated plasmin generation caused by an anti-uPA monoclonal antibody

Pro-uPA (1.3 nM) and plasminogen (85 nM) were incubated together with anti-uPA clone 1, and plasmin generation measured continuously with H-D-Val-Leu-Lys-AMC. Suramin was included in the incubations at concentrations of 0 (○), 5 (▼), 10 (▲) and 25 μg/ml (■). The lines are drawn from a mathematical model describing pro-uPA-initiated plasminogen activation using the kinetic constants previously reported for plasminogen activation in the presence of this antibody (see the Experimental section). In the absence of suramin the k_{cat}/K_m value for pro-uPA activation giving the best fit to the data is $7.6 \mu\text{M}^{-1} \cdot \text{s}^{-1}$, and this is reduced to 1.6, 1.0 and $0.28 \mu\text{M}^{-1} \cdot \text{s}^{-1}$ with the three increasing concentrations of suramin, after allowance has been made for the slightly increased rates of plasminogen activation in the presence of suramin (approx. 2-fold in these experiments). When these derived kinetic data were replotted as $(k_{\text{cat}}/K_m)^{-1}$ versus suramin concn. a K_i value for the inhibition of pro-uPA activation of 2.5 μg/ml was obtained (not shown).

tivation, and plasmin generation in this system is dependent on the cellular binding of plasminogen, we speculated that this binding may be affected by suramin. However, suramin up to a concentration of 200 μg/ml had no effect on the binding of ^{125}I -labelled [Glu¹]plasminogen to U937 cells (K_d 1.5 μM, B_{max} $9 \times 10^6/\text{cell}$), whereas a greater than 80% competition of the binding was achieved using the lysine analogue 6-amino-hexanoic acid.

The primary consequence of the cellular binding of plasminogen is that its activation by receptor-bound uPA proceeds with a greatly reduced K_m value. To determine whether suramin interfered with this despite its lack of direct effect on plasminogen binding, the kinetic constants for plasminogen activation by receptor-bound uPA were measured both in the presence and absence of suramin. Plotting of these results according to a Lineweaver–Burk plot, as shown in Figure 4, revealed that suramin indeed had a large effect on the reaction kinetics. The primary effect was on K_m , which was increased approx. 100-fold from 0.11 μM to greater than 9 μM (compared with 25–50 μM for the solution-phase reaction). Although this was accompanied by a 12-fold increase in V_{max} , reduced rates of plasminogen activation would still be apparent at plasminogen concentrations of less than approx. 1 μM. We have previously demonstrated that competition of the cellular binding of plasminogen by lysine analogues has a very similar effect on K_m , i.e. an increase to close to that of the solution-phase reaction, although with a smaller effect on V_{max} . These observations suggest that the effect of suramin is to prevent the activation of cell-associated plasminogen, and, consistent with this, in the presence of the lysine analogue 6-amino-hexanoic acid suramin gave an approx. 3-fold

Figure 4 Effect of suramin on the kinetics of plasminogen activation by receptor-bound uPA

U937 cells were pre-incubated with active, two-chain uPA, washed and incubated with various concentrations of plasminogen (up to 5 μM) in the presence (●) or absence (■) of 25 μg/ml suramin, which was shown to be a saturating concentration in preliminary experiments. Plasmin generation was measured continuously with H-D-Val-Leu-Lys-AMC as described in the Experimental section. Kinetic constants were determined from plots of V versus $V/[S]$ [34], but the data are shown here plotted as $1/V$ versus $1/[S]$, with the kinetic constants in the absence of suramin being K_m 0.11 μM, V_{max} $1.04 \text{ pM} \cdot \text{s}^{-1}$; and in the presence of suramin K_m 9.2 μM, V_{max} $12.4 \text{ pM} \cdot \text{s}^{-1}$. The inset shows the determined rate of plasminogen activation at various concentrations of suramin, plotted as in Figure 2. This gives an IC_{50} value of 2.5 μg/ml for the effect.

increase in plasminogen activation, consistent with the similar effect on the solution-phase reaction described above (results not shown).

The dependence of this effect on suramin concentration was determined by measuring rates of plasmin generation at a fixed plasminogen concentration with a range of suramin concentrations. This gave an IC_{50} value of 2.5 μg/ml (Figure 4, inset), similar to the K_i value of 1.8 μg/ml determined for the inhibition of pro-uPA activation.

To establish whether the dual effects of suramin, i.e. the direct inhibition of pro-uPA activation and the apparent inhibition of plasminogen activation, were truly independent of each other and could therefore take place concurrently, the two experiments shown in Figure 5 were performed. In the first the effect of suramin on pro-uPA-mediated plasmin generation in U937 cells was observed at plasminogen concentrations both above and below the 1 μM 'equivalence point' determined from the data in Figure 4, i.e. concentrations at which the effect of suramin on plasminogen activation should be distinctly different. As shown in Figure 5(a) at the lower plasminogen concentration both reactions were affected, giving almost complete inhibition of plasmin generation as shown previously (Figure 1), whereas at the higher plasminogen concentration the effect of suramin was restricted to pro-uPA activation, consistent with the alteration in the kinetics of plasminogen activation shown above. In the second experiment suramin was added at timed intervals after the addition of the lower concentration of plasminogen (Figure 5b). At later time points, when essentially all pro-uPA is activated, an inhibition of plasmin generation is apparent, while at time points before this, e.g. 12 min, pro-uPA activation is also inhibited, as shown by the lack of a further increase in the rate of plasmin generation. Therefore these effects are not mutually

Figure 5 Separation of the two effects of suramin on pro-uPA-mediated plasminogen activation on U937 cells

Both experiments were performed as in Figure 1, with pro-uPA pre-bound to the cells. (a) Two different concentrations of plasminogen were used, 80 nM (○, ●) and 1.5 μM (□, ■), in the absence (open symbols) or presence (closed symbols) of 25 μg/ml suramin. (b) A single plasminogen concentration (80 nM) was used and plasmin generation allowed to proceed (○) before the addition of suramin (25 μg/ml) at various times, as shown by the arrows, (●).

exclusive, and both contribute to the overall inhibition of plasmin generation elicited by suramin.

DISCUSSION

We have demonstrated here that the polysulphonated naphthyl-urea, suramin, can efficiently inhibit the activity of the uPAR-mediated plasminogen activation system, in addition to its previously described effect on the uPA-uPAR interaction [21]. The two effects described here, the non-competitive inhibition of pro-uPA activation and the interference with the activation of cell-surface-associated plasminogen, both occur with half-maximal effect at approx. 2 μg/ml, 25–100-fold lower than the concentration needed to antagonize the binding of uPA to uPAR. The possibility that these effects were not general, but in some way specific for U937 cells, is unlikely as a qualitatively similar inhibition has been observed using HT-1080 fibrosarcoma cells (V. Ellis, unpublished work). Therefore the consequence of these effects is that the generation of cell-surface-associated plasmin is completely inhibited at low concentrations of suramin.

There is currently much interest in suramin as an anti-cancer drug following initial reports of its success against certain advanced malignancies refractory to conventional therapy [1].

The mechanism of this activity is not clear although, due to its diverse effects *in vitro*, it is likely that it is mediated through more than one biological system. The uPA system of plasminogen activation is thought to be intimately involved in the processes of tumour invasion and metastasis, and the assembly of this system on the cell surface via uPAR has been proposed to be essential for its activity. Interfering with the activity of this system using anti-catalytic antibodies against uPA has been shown to inhibit tumour-cell invasion in models *in vitro* and metastasis in models *in vivo*. It is possible therefore that the anti-cancer activity of suramin *in vivo* may be due, at least in part, to inhibition of the cell-surface plasminogen activation system, as described here.

The non-competitive inhibitory mechanism of suramin on the activation of pro-uPA by plasmin is consistent with its effect on several other hydrolytic enzymes, including heparanase [8], lysosomal iduronate sulphatase and β-glucuronidase [28], and several proteases of the complement and blood coagulation systems [29], all of which include a non-competitive element in their inhibition. The specificity of this inhibition for plasmin, and not kallikrein or cathepsin G, is possibly related to the mechanism of pro-uPA activation by plasmin, which has been proposed to include interactions remote from the active site of the protease [30,31].

In contrast the effect of suramin on plasminogen activation, i.e. a specific interference with the cell-associated but not the solution-phase reaction, is not due to an inhibition of the catalytic reaction *per se*, but rather to a reversal of the favourable kinetic changes which occur as a consequence of the cellular binding of plasminogen [13]. The effect is therefore similar to that observed previously with analogues of lysine, which exert their action by antagonizing the cellular binding of plasminogen due to occupation of the lysine-binding sites on certain of its kringle domains. Suramin, however, appears to exert its effect through a different mechanism, as it neither directly interfered with the lysine-binding sites of plasminogen, as demonstrated by the conservation of the ability of the anti-uPA clone 1 monoclonal antibody to potentiate plasminogen activation via a lysine-binding-site-dependent mechanism, nor inhibited the cellular binding of ¹²⁵I-labelled plasminogen.

The mechanism by which cell-associated plasminogen is activated by receptor-bound uPA has not yet been fully elucidated, particularly with respect to the nature and role of the heterogenous group of molecules responsible for the binding of plasminogen, although various models have been proposed to account for their involvement [11,20]. Based on these models two distinct mechanisms for the inhibitory effect of suramin could be envisaged. First, that it specifically inhibits the interaction of plasminogen with a discrete subpopulation of binding molecules, constituting the functionally important sites for the activation of plasminogen. This mechanism would require that these sites are of such low abundance, relative to the total plasminogen-binding capacity of the cells (9×10^6 molecules/cell), that blocking them escapes detection in the radioligand-binding assays, which would certainly be the case if these sites were of similar abundance to uPAR (1.5×10^4 /cell, results not shown [32]). Secondly, suramin could exert its effect by interfering with the interaction between uPAR-bound uPA and cell-bound plasminogen. As an alternative to a direct interference between the proteases this could also be envisaged to occur due to interference with a putative interaction between the plasminogen-binding molecules and receptor-bound uPA or uPAR itself [11]. Which, if any, of these mechanisms is responsible for the observed effects cannot be resolved without further elucidation of the functional assembly of this system of plasminogen activation, and suramin itself may prove to be a useful tool in these studies.

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