

The Ability of Suramin To Block CD4-gp120 Binding Is Reversed in the Presence of Albumin

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We have shown that suramin can directly inhibit the binding of the human immunodeficiency virus type 1 gp120 envelope protein to immobilized CD4, thus helping to explain the previously described antiviral properties of suramin. However, physiological concentrations of serum albumin significantly attenuated suramin's antiviral effects, suggesting that only free suramin has antiviral properties. Reported discrepancies between in vitro and clinical activities of suramin may be due to differences between free suramin levels in experimental assays and those achievable clinically.

Suramin, a polyanionic compound synthesized in 1920, has been used for decades in the treatment of African trypanosomiasis (sleeping sickness). Suramin can inhibit the reverse transcriptase (RT) of RNA tumor viruses and possesses potent anti-human immunodeficiency virus (HIV) activity in vitro (11). However, clinical trials of this compound in patients with AIDS failed to show significant benefit (3, 4, 8).

No clear explanation has been offered for the discrepancy between the in vitro activity of suramin and the clinical trial results. Speculations have included a suramin-related immunosuppressive effect and failure of the drug to adequately penetrate the central nervous system (4). It has been recognized that the drug's long serum half-life (5) is related to its ability to bind to serum albumin (12). We realized that in vitro assays of suramin's antiviral activity were performed at subphysiological albumin concentrations. Although the suramin concentration used was comparable to that achievable clinically, differences in albumin levels between in vivo and in vitro situations could have resulted in significant differences in concentrations of free suramin. We therefore investigated whether the antiviral effects of suramin might be attenuated in the presence of albumin.

The MT-4 CD4-positive lymphocyte cell line (6) was cultivated at 37°C under 5% CO₂ as described previously (14) and was used to propagate the HIV-III_B strain of HIV-1 (kindly supplied by R. C. Gallo, Bethesda, Md.). Viral infectiousness was quantified by endpoint titration in MT-4 cells. Viruses were frozen at -70°C until use. Suramin was obtained from FBA Pharmaceuticals, New York, N.Y. Dextran sulfate (molecular weight, 6,000 to 8,000) was obtained from ICN Chemicals, Cleveland, Ohio. Both drugs were dissolved in phosphate-buffered saline (PBS) (10 mg/ml) and frozen at -20°C until use. Bovine serum albumin and human serum albumin (Sigma Co., St. Louis, Mo.) were each stored at -20°C at 100 mg/ml in PBS. MT-4 cells (5 × 10⁵ cells/ml) were infected with HIV-III_B (0.1 50% tissue culture infective dose per cell) for 2 h. Albumin and antiviral compounds were added to the cell culture concomitant with virus. Cells were washed three times after infection and cultured in the presence or absence of albumin and antiviral compounds. After 4 days, supernatants and cells were

harvested; RT assays and indirect immunofluorescence assays using anti-HIV p24 antibody were performed as described elsewhere (7, 14).

CD4 receptor-gp120 binding assays were performed by using a CD4-gp120 capture enzyme-linked immunoadsorption assay (American Bio-Technologies Inc., Boston, Mass.). Briefly, 100 μl of HIV-1 gp120 (ABI Biotechnology, Rockville, Md.) (100 ng/ml) was placed into wells coated with CD4 receptor in the presence of different concentrations of suramin and/or albumin and incubated for 1 h at room temperature. Wells were washed, and mouse anti-gp120-peroxidase conjugate (1:25) was added to the wells for 1 h; this was followed by further washing. Finally, 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Md.) was added, and colorimetric reactions were read at a wavelength of 450 nm.

Inhibition of CD4-gp120 binding. The ability of suramin to interfere in a concentration-dependent fashion with the binding of gp120 to CD4 is illustrated in Fig. 1. Also shown is the attenuation of this effect in the presence of bovine albumin. Similar results were obtained in each of three different experiments.

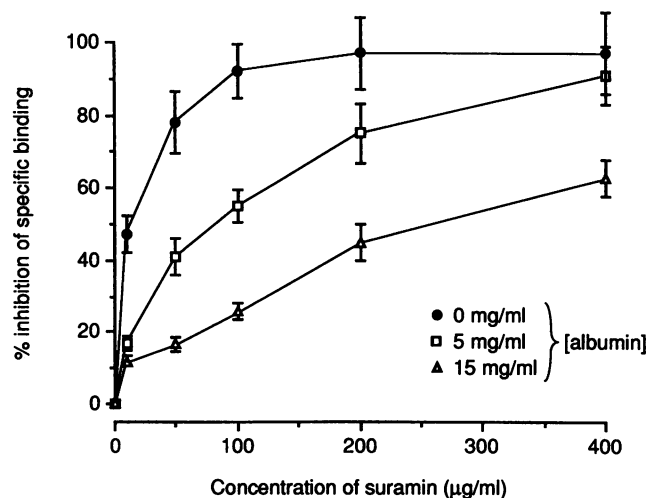


FIG. 1. Effect of bovine albumin on suramin blockade of gp120-CD4 binding. Bars indicate standard errors.

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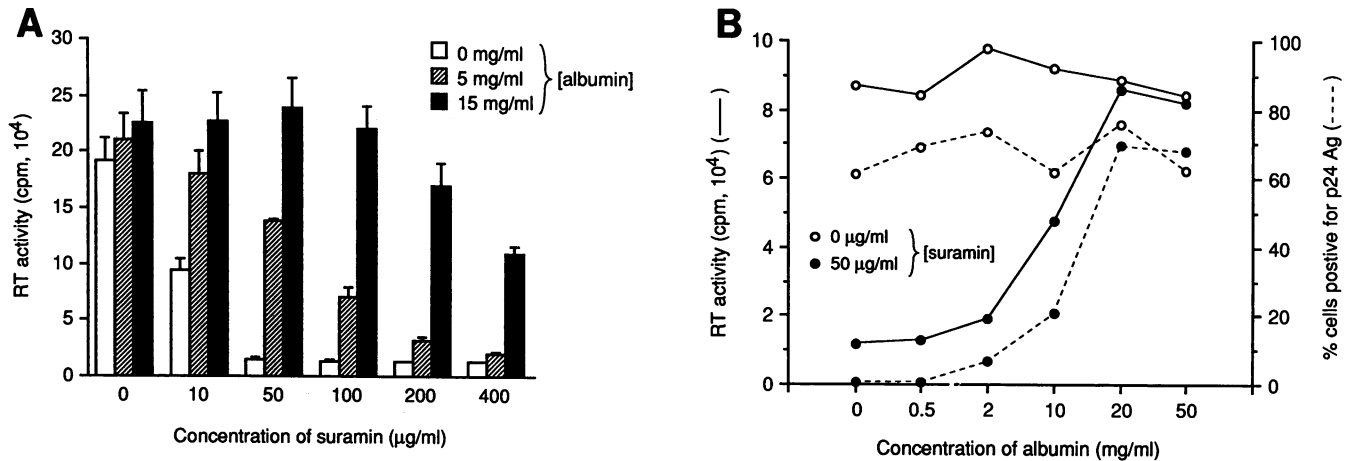


FIG. 2. Influence of albumin on the antiviral effect of suramin. (A) Effect of bovine albumin with regard to generation of RT activity in infected cells. Bars indicate standard errors. (B) Effect of human albumin on p24 antigen (Ag) generation (—) and RT activity (---) in infected cells.

Attenuation of antiviral effect by albumin but not dextran sulfate. Figure 2A shows one of three experiments which demonstrated that exposure of HIV-1-infected MT-4 cells to clinically relevant concentrations of suramin (200 to 300 mg/liter) resulted in significant inhibition of RT activity, which is consistent with previous observations made with other cells (11). This inhibitory effect was attenuated in the presence of increased bovine albumin concentrations. This effect was pronounced when the ratio of albumin molecules to suramin molecules exceeded 1:1 (data not shown), consistent with reports that there are one or two suramin-binding sites per albumin molecule (12). The results presented in Fig. 2B show that human serum albumin exerted a similar antisuramin effect with regard to expression of both HIV RT and p24 antigen. Data of this nature were obtained in each of two experiments which employed human albumin concentrations as high as 50 mg/ml, i.e., within the physiological range.

We also examined the effect of bovine albumin on the activity of dextran sulfate, another agent reported to inter-

fere with binding of HIV-1 to the CD4 receptor (1, 10). Table 1 shows the results of one of two studies performed which showed that the effect of bovine albumin on suramin antiviral activity was specific, in that the activity of dextran sulfate was unaffected. This is consistent with our hypothesis that the ability of albumin to attenuate the antiviral activity of suramin is due to a specific albumin-suramin interaction.

The mechanisms by which suramin acts to interfere with CD4-gp120 binding remain obscure, but this effect may be due to a nonspecific action of this polyanionic drug either on the conformation of proteins in solution or on cell membranes. Suramin also inhibits the binding of a number of peptide growth factors to their receptors (2, 13), and these activities are also attenuated in the presence of albumin (13). Although clinical trials of suramin for the treatment of AIDS did not find the drug to be efficacious, toxicities were nonetheless noted (3, 4, 8) and may be attributable to albumin-suramin complexes rather than to free drug, since cellular uptake of such complexes has been reported (9).

The CD4-gp120 interaction remains an important target for HIV-1 therapy. Suramin has been reported to inhibit binding between anti-gp120 antibody and gp120-expressing HUT-78 cells, although with reduced efficacy in the presence of 10% serum (15); these findings are consistent with our own observations regarding CD4-gp120 interactions and the effect of albumin on suramin's antiviral activity.

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TABLE 1. Effect of albumin on inhibitory activity of suramin and dextran sulfate in MT-4 cells infected by HIV-1^a

Inhibitory agent and concn (μg/ml)	% p24-positive cells at an albumin concn (mg/ml) of:			RT activity (cpm/ml [10 ³]) at an albumin concn (mg/ml) of:		
	0	5	10	0	5	10
Suramin						
0	62	60	57	13.7	10.1	10.4
10	35	51	51	7.5	7.6	8.3
50	0	48	49	0.6	4.8	6.8
100	0	2	15	0.4	0.9	3.2
Dextran sulfate						
0	62	60	57	13.7	10.1	10.4
10	0	0	0	0.9	0.9	1.3
50	0	0	0	0.8	0.7	1.1
100	0	0	0	0.9	1.2	0.5

^a Cells were incubated with HIV-1 (0.1 50% tissue culture infective doses per cell), albumin, and either suramin or dextran sulfate for 2 h, washed, and cultured for 4 days in the presence of albumin and either suramin or dextran sulfate prior to p24 immunofluorescence assay and RT assay.

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