The inhibitory effects of suramin on HIV-1 are attenuated in the presence of albumin

Suramin has previously been observed to inhibit *in vitro* infectivity and replication of retroviruses, including that of HIV-1 [1]; however, clinical trials have shown little efficacy in the treatment of patients with AIDS [2]. We report a cell-free system where physiological concentrations of serum albumin significantly reduced the antiviral activities of suramin in a concentration-dependent manner.

The long serum half-life of suramin has been attributed to its ability to bind strongly to serum albumin [3]. Although total suramin concentrations used in *in vitro* assays are comparable to those achievable clinically [2], the differences between physiological levels of albumin and those present in laboratory assays could have resulted in differing concentrations of free suramin.

This work was carried out with the MT-4 CD4+ lymphocyte cell line [4] and the IIIB laboratory strain of HIV-1 [5], as previously described [6]. Suramin (FBA Pharmaceuticals, New York, New York, USA) was dissolved in phosphate-buffered saline (PBS) at 10 mg/ml and frozen at −20°C until use. Bovine albumin (Sigma Chemical Co., St Louis, Missouri, USA) was stored at −20°C at a concentration of 100 mg/ml in PBS.

MT-4 cells were suspended at 0.5×10^6 cells/ml and infected with HIV-I_{IIIB} at 0.1 tissue culture infective dose ($TCID_{50}$)/cell for 2 h. Antiviral compounds and albumin were added into cell culture simultaneous with infection, or 2 h postinfection. Cells were washed three times after infection and cultured in medium in the presence or absence of antiviral compounds and albumin. After 4 days of culture, the supernatants and

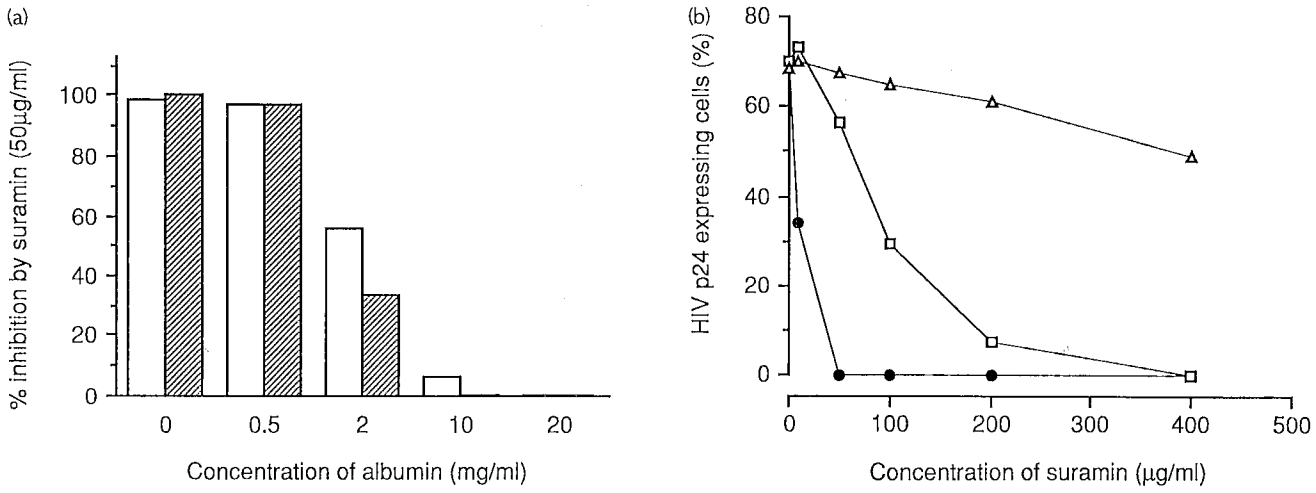

Fig. 1. Influence of albumin on inhibitory effect of suramin on HIV replication and reverse transcriptase (RT) activity in MT-4 cells. (a) Exposure of cells to compounds during and following HIV infection. Cells were infected with HIV-1 [0.1 tissue culture infective dose ($TCID_{50}$)/cell] for 2 h in the presence of suramin (50 µg/ml) and varying concentration of albumin; washed, and cultured in media containing the same concentration of the compounds. At day 4, RT activity and HIV-p24 expressing cells were measured; □, % inhibition of RT; ▨, % inhibition of p24-positive cells. (b) Experiment was carried out similarly to above, except exposure to various concentrations of albumin and suramin was restricted to the 2 h period of viral absorption; albumin: ●, 0 mg/ml; □, 10 mg/ml; △, 30 mg/ml.

cells were harvested; reverse transcriptase (RT) assays and indirect immunofluorescence assays (IFA) using anti-HIV p24 antibody were carried out as previously described [6].

Figure 1 shows that both 2 h and 4 day exposure of HIV-1-infected MT-4 cells to suramin (50 µg/ml) resulted in inhibition of production of RT activity into culture fluid and a decrease in the percentage of cells expressing HIV p24. These results are consistent with previous observations which used different experimental systems [1,2]. However, the inhibitory effect of suramin was progressively reduced in the presence of increasing concentrations of albumin. At albumin concentrations close to those found in serum (approximately 3.5 mg/l), the inhibitory effect was almost eliminated (Fig. 1a). Clinically achievable serum suramin levels that can be tolerated without dangerous toxicity are in the 200–300 mg/l range. Figure 1b shows the profound effect of albumin on suramin inhibition of p24 expression at this concentration of drug. Conversion of these concentrations to molar terms (data not shown) reveals that attenuation of the suramin effect was marked when the ratio of albumin-to-suramin molecules exceeded 1 : 1, consistent with reports that there are between one and two suramin binding sites per albumin molecule [3].

Our data show that the impressive *in vitro* antiviral effects of suramin are attenuated in the presence of albumin. Strong binding between suramin and albumin has previously been demonstrated, and suramin has been estimated to be >99% protein-bound in serum [3]. While it has been established that albumin–suramin binding is relevant to suramin pharmacokinetics, underlying the long half-life of the drug, the effect of this binding on the bioavailability of suramin has not previously been studied. Previous *in vitro* studies that detected antiviral activity of suramin employed subphysiological albumin levels.

We conclude that only free suramin has antiviral properties, and that the discrepancy between *in vivo* and *in vitro* activity of suramin is due, at least in part, to the difference in free suramin levels achievable clinically and those used in standard *in vitro* assay systems.

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References

1. DE CLERCQ E: Suramin: a potent inhibitor of the reverse transcriptase of RNA tumor viruses. *Cancer Lett* 1979, 8:9–22.
2. KAPLAN L, WOLFE P, VOLBERDING P, *ET AL.*: Lack of response to suramin in patients with AIDS and AIDS-related complex. *Am J Med* 1987, 82:615–620.
3. VANSTERKENBURG E, WILTING J, JANSSEN L: Influence of pH on the binding of suramin to human serum albumin. *Biochem Pharmacol* 1989, 38:3029–3035.
4. HARADA S, KOYANAGI Y, YAMAMOTO N: Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. *Science* 1985, 229:563–565.
5. GALLO RC, SALAHUDDIN SZ, POPOVIC M, *ET AL.*: Frequent detection and isolation of cytopathic retroviruses (HTLV-III)

from patients with AIDS and at risk for AIDS. *Science* 1984, 224:500–503.

6. TREMBLAY M, NUMAZAKI K, LI X-G, *ET AL.*: Resistance to infection by HIV-1 of peripheral blood mononuclear cells from HIV-1-infected patients is probably mediated by neutralizing antibodies. *J Immunol* 1990, 145:2896–2901.