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 × 10⁶ cells/ml and infected with HIV-1IIIB at 0.1 tissue culture infective dose (TCID₅₀)/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
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 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 sub-physiological albumin levels.

We conclude that only free suramin has antiviral properties, and that the discrepancy between in vitro 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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