The anti-parasitic drug suramin potently inhibits formation of seminal amyloid fibrils and their interaction with HIV-1

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Running title: Suramin is a seminal amyloid inhibitor

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**ABSTRACT**

Seminal amyloid fibrils are made up of naturally occurring peptide fragments and are key targets for the development of combination microbicides or antiviral drugs. Previously, we reported that the poly-sulfonic compound ADS-J1 is a potential candidate microbicide that not only inhibits HIV-1 entry, but also seminal fibrils. However, the carcinogenic azo moieties in ADS-J1 preclude its clinical application. Here, we screened several ADS-J1-like analogs and found that the antiparasitic drug suamin most potently inhibited seminal amyloid fibrils. Using various biochemical methods, including Congo red staining, CD analysis, TEM, viral infection assays, surface plasmon resonance imaging, and molecular dynamics simulations, we investigated suamin’s inhibitory effects and its putative mechanism of action. We found that by forming a multivalent interaction, suamin binds to proteolytic peptides and mature fibrils, thereby inhibiting seminal fibril formation and blocking fibril-mediated enhancement of viral infection. Of note, suamin exhibited potent anti-HIV activities, and combining suamin with several antiretroviral drugs produced synergistic effects against HIV-1 in semen. Suramin also displayed a good safety profile for vaginal application. Moreover, suamin inhibited the semen-derived enhancer of viral infection (SEVI)/semen-mediated enhancement of HIV-1 transcytosis through genital epithelial cells and the subsequent infection of target cells. Collectively, suamin has great potential for further development as a combination microbicide to reduce the spread of the AIDS pandemic by targeting both viral and host factors involved in HIV-1 sexual transmission.

**INTRODUCTION**

Acquired immune deficiency syndrome (AIDS) remains a global pandemic because the annual incidence of new infection has stayed relatively constant and the number of HIV/AIDS patients continues steadily increased despite the successful application of antiretroviral (ARV) therapy (1). The majority of new HIV-1 infections come from sexual intercourse, during which semen plays critical roles in transmitting HIV-1. Semen contains various cationic amyloid fibrils that are made up of naturally occurring peptide fragments, including prostatic acid phosphatase (PAP248-286 and PAP85-120) and the homologous proteins semenogelin 1 (SEM1) and semenogelin 2 (SEM2) (2-4). Among them, PAP248-286 self-aggregates to form an amyloid fibril named semen-derived enhancer of viral infection (SEVI), which is the best characterized seminal amyloid (3). These proteolytic fragments contain several basic residues, which renders these fibrils very cationic with the ability to capture HIV-1 virions, promote viral attachment to target cells, and augment viral fusion (3). These amyloid fibrils also counteract the antiviral efficacy of a panel of ARV drugs (5).
Chemicals that could antagonize the effects of SEVI fibril on HIV-1 infection enhancement might be attractive adjunctive agents in the development of topical treatment to stop HIV-1. Previously, we reported that ADS-J1, an anionic HIV-1 entry inhibitor, also displayed efficacy in inhibiting seminal amyloid fibril formation and antagonizing the ability of the fibrils to enhance HIV-1 infection (6). Particularly, ADS-J1 displayed synergetic effects with ARV drugs on inhibiting viral infection in semen. This dual-functional agent, which targets both virus and seminal amyloid fibrils, represents an alternative option to design a combination candidate microbicide. However, the azo moieties in ADS-J1 are unstable and carcinogenic, which impair its further advancement into clinical trials (7). Thus, we screened several ADS-J1-like polysulfonic chemicals, in an attempt to find a chemical that is clinically practical as a dual-functional candidate microbicide. In this screen, we identified suramin.

Suramin is a hexasulfonated naphthylurea compound that is a market-authorized drug used for antiparasitic treatment since 1921. However, suramin possesses miscellaneous pharmacological effects, including antitumor (8,9), anti-inflammation (10) and antivirus activities (11-14) etc. Suramin was the first documented HIV-1 reverse transcriptase inhibitor tested in clinical experiments; however, the side effects of the systemic drug delivery of this drug over a long term treatment resulted in the failure of these clinical trials (15). In this study, we explored the effects of suramin on inhibiting seminal amyloid fibrils and the underlying mode of action. We also evaluated the potential of suramin to be developed as an adjunctive agent with current ARV drugs. Additionally, the potential of suramin to prevent HIV-1 from transcytosis through genital epithelial cells and to prevent the subsequent infection of target cells, as well as, its safety profile for vaginal use, was investigated.

RESULTS

Suramin inhibits semen-derived amyloid fibril formation

In an attempt to indentify an active compound that acts as a multifunctional microbicide with safer profile than ADS-J1, we noticed a series of commercially available suramin-like compounds with similar molecular weights, and degrees of sulfation, to ADS-J1, but without clear harmful bonds (Figure 1A-G). The thioflavin T (ThT) assay and the Congo red binding assay are two fast and conventional methods used to detect amyloid fibrils. Because several of these compounds could induce an increase in the fluorescence intensity of ThT, we applied the Congo red binding assay to test their inhibitory effects on PAP248-286 aggregation. The fibril formation induced by our synthetic PAP248-286 was easily observed in the absence of inhibitors. We found that suramin possessed excellent potency to inhibit PAP248-286 aggregation since it induced the lowest Congo red optical absorbance at all tested time points among the tested compounds (Figure 1H). Therefore, we performed in-depth research to characterize the inhibitory effects of suramin and the putative
mechanism of action.

We validated the inhibitory effect of suramin on PAP248-286 fibrillization using distinct biochemical methods. The substantial loss of the lag-phase of PAP248-286 in the presence of suramin suggested limited fibril formation (Figure 2A). Notably, suramin could completely inhibit SEVI fibril formation within 48 h at a peptide:suramin concentration of 1:2 (molar ratio). Circular dichroism (CD) spectra revealed that suramin interfered with the β-sheet formation of PAP248-286. PAP248-286 began to form a peak at 222 nm, a characteristic feature of β-sheet structure, at the time of 4 h. However, in the presence of suramin, PAP248-286 did not show signs of β-sheet structure at the times of both 4 h and at 24 h (Figure 2B). Transmission electron microscopy (TEM) analysis also confirmed that suramin prevented the aggregation of PAP248-286 into SEVI fibrils. At 4 h, PAP248-286 had already formed amyloid fibrils. At the time point of 24 h, abundant mature fibrils could be detected. However, in the presence of suramin, SEVI fibrils could not be detected at 4 h and at 24 h (Figure 2C). Additionally, using a viral infection assay, we characterized the ability of PAP248-286 samples to enhance HIV-1 infection after agitation with or without suramin for various times. To eliminate the anti-HIV activity of suramin, the above samples were first centrifuged at 12,000 rpm for 5 min to remove free suramin. This assay revealed that PAP248-286 alone enhanced the HIV-1 infection by three HIV-1 clones in a time-dependent manner, indicating that PAP248-286 formed amyloid fibrils over time. However, the ability of PAP248-286 to enhance HIV-1 infection was decreased after agitation in the presence of suramin in a dose-dependent manner. In the presence of 2-fold excess suramin, the PAP248-286-induced enhancement of HIV-1 infection was almost completely abolished. We also analyzed a control, in which suramin at the highest concentration (3520 μM, 8 times the concentration of PAP248-286) was tested at the time point of 48 h in the absence of PAP248-286, to determine the inhibitory effects of suramin on HIV-1 infection. We found that under these conditions, suramin did not show inhibitory effects on HIV-1 infection, suggesting that limited nonspecific binding of suramin with the incubation tube that might be transferred to the infection system (Figure S1). The half-maximal inhibitory concentration (IC₅₀) of suramin against the fibrillization of 440 μM PAP248-286 was approximately 79.52 μM (Figure S2). Similar results were obtained in seminal fluid (SE-F) samples. Figure 2E shows that fresh SE-F samples displayed increased HIV-1 infection after an 8-hour agitation with three infectious HIV-1 clones, indicating the formation of new seminal fibrils within experimental period. Notably, suramin inhibited the formation of new seminal fibrils in fresh SE-F, thus inhibiting the fibril-mediated enhancement of viral infection. In this infection assay, SE-F was first incubated with suramin, and the sample was then centrifuged to remove the remaining free suramin to avoid any direct activity of suramin against HIV. Interestingly, the same SE-F sample showed a distinct ability to enhance the infection of different HIV-1
strains (Figure 2E).

We also investigated the inhibitory effects of suramin on SEM1(86-107) aggregation, another amyloidogenic peptide found in semen. Suramin at a high concentration also possessed antiaggregation ability against SEM1(86-107) (Figure 3). However, the inhibitory effect of suramin on SEM1(86-107) was weaker than the inhibitory effect of suramin on PAP248-286. This difference was likely due to the lower number of cationic residues in SEM1(86-107) than in PAP248-286 (16). Specifically, we found that, unlike the polyanions that have failed as candidate microbicides in the clinical stage (17), suramin did not facilitate SEVI fibril formation at low concentration, while cellulose sulfate at the same concentration promoted PAP248-286 fibril aggregation (Figure 4). Taken together, our findings suggested that suramin effectively inhibited seminal amyloid fibrils formation.

**Suramin specifically binds to PAP248-286 to prevent peptide assembly**

We next set out to characterize the molecular interactions between suramin and PAP248-286, which might be vital for suramin’s inhibitory effects on PAP248-286 aggregation. A mutant PAP248-286(Ala), in which the eight positively charged lysine residues and arginines in PAP248-286 were replaced with neutral alanine residues, was assessed to investigate the interaction between suramin and PAP248-286. Suramin bound to PAP248-286 but not to PAP248-286(Ala), as evidenced by the observation after the coincubation of suramin with each peptide, the level of PAP248-286 in the supernatant gradually decreased with increasing amounts of suramin (Figure 5A, left panel; Figure S3), whereas the level of PAP248-286(Ala) in the supernatant did not change with or without suramin (Figure 5B, Figure S3). Polybrene, a cationic polymer, antagonized the binding ability of suramin to PAP248-286 (Figure 5A, right panel, Figure S3). In agreement with the above results, suramin did not inhibit PAP248-286(Ala) assembly even at the concentration of eight times greater than the concentration of PAP248-286 (Figure 5C), possibly due to the inability of suramin to interact with PAP248-286(Ala). Taken together, these results suggest that the electrostatic interaction between suramin and PAP248-286 is fundamental for suramin to inhibit PAP248-286 aggregation.

We next determined the binding affinity between each suramin-analog and PAP248-286. The binding capacity of six compounds to PAP248-286 was diverse, and suramin had the strongest binding ability to PAP248-286 among the tested compounds (Figure 5D). For clarity, only three compounds, suramin, NF157, and NF449, representing of strong, moderate, and weak SEVI inhibitors, respectively, were chosen to further determine the binding affinity by surface plasmon resonance imaging (SPRi). The binding affinity (K_D) of suramin, NF157 and NF449 with PAP248-286 were calculated to be 1.18×10^{-6} M, 3.19×10^{-6} M and 1.52×10^{-5} M, respectively (Figure 5E). We further explored the molecular details of the interactions between the suramin analogs and PAP248-286 by molecular
dynamics (MD) simulation. The MD simulation showed that eight suramin molecules bind to PAP248-286, completely covering the surface of the peptide. Seven NF157 molecules interacted with PAP248-286 and only five NF449 molecules combined with PAP248-286, partly covering the peptide (Figure 5F). All three compounds had electrostatic interactions with PAP248-286. In addition to electrostatic interactions, hydrogen bonds and hydrophobic interactions also existed in the protein-molecule complexes. Suramin formed the most interactions with all three types, followed by NF157, and NF449 (Figure 5G). These MD results nicely agree with the SPRi results. Suramin exhibited a good binding affinity toward PAP248-286, in accordance with its high potency to inhibit SEVI fibril formation.

**Suramin blocks the interaction of amyloid fibril with HIV-1 and diminishes the fibril-mediated enhancement of viral infection**

The cationic property of seminal fibrils contributes to their abilities to enhance HIV-1 infection. Suramin might also interact with mature amyloids, abrogating the fibril-mediated enhancement of HIV-1 infection. Zeta potential determination showed that suramin neutralized, and even reversed the positive surface charge of SEVI fibrils (Figure 6A). SEVI alone bound more than 60% of the input virus, whereas SEVI gradually lost the ability to bind to the virus with the addition of increasing concentrations of suramin (Figure 6B). Confocal microscopy images revealed that SEVI fibril alone (Figure 6C) or agitated SE-F (Figure 6E) could effectively sequester eGFP-HIV-1. In contrast, in the presence of suramin, SEVI and SE-F was unable to form fibril-virus complexes (Figure 6C and E), which indicated that suramin markedly inhibited the capture of HIV-1 virions by SEVI- or semen-derived amyloid fibrils. As expected, SEVI and 2.5% fresh SE-F, which has been agitated for 8 h to allow new amyloid fibrils to form, enhanced the HIV-1 infection of different viral strains by 21- to 146-fold and 2- to 16-fold in the absence of suramin, respectively. However, the addition of suramin decreased the SEVI- or SE-F-induced enhancement of infection in a dose-dependent manner, indicating that suramin abolished the SEVI- and SE-F-mediated enhancement of HIV-1 infection (Figure 6D and F).

**Combination of suramin with ARV drugs shows synergistic effects against HIV-1 infection in semen**

Suramin has anti-HIV activity (18-20). We also assessed the direct inhibition of HIV-1 SF162 and HIV NL4-3 infection by suramin alone, and the IC$_{50}$ values were 3.17 μM and 0.21 μM, respectively (Figure S4). Although it has been reported that SE may diminish the efficacy of several ARVs (5), suramin could still completely suppressed the viral infection of HIV-1 SF162 and HIV NL4-3 in SE-F with slightly increased IC$_{50}$ values of 15.13 μM and 0.66 μM, respectively, compared to that IC$_{50}$ in the absence of SE-F (Figure S4). Herein, we found that suramin is also a seminal amyloid inhibitor. We next assessed the potential cooperative effects of suramin combined with several ARV-based candidate microbicides on the infection of HIV-1 SF162 and HIV-1 NL4-3 in
Selected ARV drugs included maraviroc, azidothymidine (AZT), nevirapine, raltegravir, and TMC120. As shown by the data in Table 1, the combination of suramin and maraviroc resulted in strong synergism (CI$_{50}$:0.233). The combination of suramin with nevirapine, raltegravir, or TMC120 exhibited synergistic effect (CI$_{50}$:0.614-0.653), and the combination of suramin with AZT showed moderate synergism (CI$_{50}$:0.739) against HIV-1 SF162. The synergistic effects of these combinations were also observed on HIV-1 NL4-3 infection (Table 2). The combination of suramin with AZT, nevirapine, or TMC120 resulted in synergism (CI$_{50}$:0.496-0.646). The combination of suramin with raltegravir displayed moderate synergism (CI$_{50}$:0.704).

**Suramin is safe for vaginal use in rabbits**

Safety is a key determinant to evaluate a candidate microbicide. The data in Table 3 and Figure S5 indicated that suramin showed very low or no in vitro cytotoxicity to various vaginal and cervical epithelial cell lines, colorectal carcinoma cells and lymphocytes with CC$_{50}$ values all being greater than 1000 μM, which was more than 60 times the inhibitory concentration on PAP248-286 fibrillization at its physical concentration (~8 μM) (3) and 290 times greater than the IC$_{50}$ against HIV-1 SF162 infection. Subsequently, we performed an in vivo safety evaluation of suramin gel on rabbits. The intravaginal use of nonoxynol-9 (N-9), a nonionic detergent, was found to injure epithelial cell membranes, resulting in the release of pro-inflammatory cytokines, recruitment of inflammatory mediators and efflux of macrophages in the vagina (21). Therefore, in our study, an N-9 gel was used as a negative control, and a placebo gel used as a positive control. The intravaginal administration of 1% or 4% suramin gel did not result in significant microscopic abnormalities (Figure 7A). The morphological alterations noted in the vaginal epithelium and submucosa were similar to those observed in the placebo gel. The suramin gel-treated group retained the structural integrity of the vaginal epithelium, in which the lamina propria and squamous epithelium appeared to be intact. The absence of leukocyte infiltration in the representative upper and lower regions of the rabbits’ vaginal tissues was observed. In contrast, the N-9 gel-treated group showed multifocal damage of the vaginal mucosa characteristic of congestion, edema, inflammatory infiltration, and epithelial disruption (Figure 7A).

PCNA expression was detected by an immunohistochemical method (Figure 7B and Table 4). Although a slight increase in PCNA staining was observed in the epithelium of suramin-treated tissues, no significant differences in PCNA staining were noted in the stromal cells of the placebo control-treated and suramin-treated tissues.

The levels of various cytokines (IL-6, IL-8, TNF-α, and IL-10) in cervicovaginal lavages (CVLs) of suramin-treated rabbits were not significantly different, from placebo controls (Figure 7C-F). However, the N-9 gels induced a marked inflammatory reaction (Figure 7C-F).
Suramin prevented the SEVI- and SE-F-mediated enhancement of HIV-1 absorption, transcytosis through genital epithelial cells and subsequent infection of target cells

The vaginal and cervical epithelia provide an initial barrier to infection from sexually acquired HIV-1 in women. In an attempt to evaluate the inhibitory effects of suramin on HIV-1 transmission under conditions that more closely mimic that in vivo condition, we examined the effects of suramin on HIV-1 translocation across human cervical epithelial cells HEC-1A in the presence of SEVI or SE-F using a Transwell culture system.

We first used a cell binding assay to confirm the inhibitory effects of suramin on the absorption of HIV-1 into HEC-1A cells. 10 μM SEVI fibrils (Figure 8A) or 2.5% of SE-F that was agitated for 8 h to allow the formation of new fibrils (Figure 8B) and HIV-1 were first incubated with or without 40 μM suramin at 37 °C for 30 min. Then, the mixtures were centrifuged for 10 min at 5000 rpm. The virions in the supernatant were added to a confluent HEC-1A monolayer that was cultured for 4–7 days. After an 8-hour incubation, the cells were washed with PBS and then lysed to quantify HIV-1 p24 by Western blot. HEC-1A cells bound or internalized fewer virions in samples treated with SEVI (Figure 8A) and SE-F (Figure 8B) than the virus control samples. However, suramin prevented the binding of amyloid fibrils with HIV-1, and, the SEVI- or SE-F- treated samples showed increased bound or internalized virions in HEC-1A cells exposed to suramin (Figure 8A and B) compared to those without suramin.

Next, we applied suramin, SEVI or SE-F and HIV-1 to a HEC-1A cell monolayer growing in the upper compartment of a dual-chamber system, to evaluate the inhibitory activity of suramin on the absorption and transcytosis of HIV-1 through epithelial cells in the presence of seminal amyloid fibrils and on the subsequent infection of target cells in the bottom chamber (Figure 8C). After an 8-h incubation with suramin, SEVI/SE-F and HIV-1 on the apical surface of the HEC-1A monolayer, the culture medium were removed, and the HEC-1A cells were washed to remove unbound virus, detached and lysed. Then, the amounts of attached virus were determined by Western blot of HEC-1A lysates. TZM-bl cells in the bottom chambers continued to incubate for 48 h and were then subjected to luciferase assay. We found that SEVI (Figure 8D and E) and SE-F (Figure 8F and G) could enhance the binding or internalization of HIV-1 in HEC-1A cells (Figure 8D and F) and the subsequent infection of TZM-bl cells (Figure 8E and G), respectively. However, the level of bound or internalized HIV-1 in HEC-1A cells and the infected TZM-bl cells were decreased in the suramin-treated samples compared to the SEVI-treated or SE-F-treated samples. These results suggested that suramin inhibited the transcytosis of HIV-1 across genital epithelial cells in the presence of seminal amyloid fibrils and the subsequent infection of lymphocytes in the bottom chamber. Of note, SE-F at the concentration used in this study showed limited cytotoxicity on both TZM-bl cells and HEC-1A cells (Figure S6).
DISCUSSION

Suramin is a well known anti-HIV-1 agent. However, in this study, we revisited its potential to prevent HIV-1 sexual transmission, focusing not on the HIV-1 itself, but on the host factor, seminal amyloid fibrils, which are exploited by the virus to facilitate its transmission and escape from ARV treatment (3,5). Suramin was shown here to be a potent seminal amyloid inhibitor. Suramin binds to amyloidogenic peptide and inhibits peptide aggregation into fibrils (Figure 2). Moreover, suramin interacts with mature fibrils and blocks the fibril-mediated enhancement of viral infection (Figure 6).

The cationic property of seminal amyloid fibrils is an important element to consider when designing an amyloid inhibitor (22). Owing to their multiple cationic residues, amyloidogenic peptide and mature fibrils, both of which naturally occur in semen (2,4,23), putatively display multiple, identical and periodically spaced binding sites for an anionic agent. The anionic chemicals could simultaneously bind to two or more tethered cationic domains in a peptide or fibril via multivalent interaction, creating a significant steric barrier to block amyloidogenic peptide aggregation and the interactions between fibril and virus. As expected, suramin shows excellent potential as a seminal fibril inhibitor because it could target both the free amyloidogenic peptide to inhibit peptide aggregation and the mature fibrils to block the interaction between virus and fibrils. Compared to other agents that target only amyloidogenic peptide or mature fibril (24-26), this dual-functional effect on amyloid fibril makes suramin a practical candidate microbicide.

The results from the binding assays and MD simulation suggested that the different binding capability of suramin-analogs to PAP248-286 would translate into varied efficacy for inhibiting SEVI fibril formation. First, electrostatic interactions are essential for the ability of suramin to inhibit seminal fibril formation, since its inhibitory effect was markedly reduced by the mutation of the cationic residues in PAP248-286. Additionally, the binding capacity of suramin was lost in the presence of a cationic polymer or with the neural mutant peptide (Figure 5).

The relocation of the sulfonate groups to other positions in the suramin structure led to the partial loss of activity, such as NF279 and NF449 (Figure 1). Second, the hydrogen bonding capability of secondary amide groups and the hydrophobic interaction involving other parts of the molecule are also likely to be important. MD simulation revealed that hydrophobic bonds account for more than half of the interactions between suramin analogs and PAP248-286 (Figure 5G). Compared to suramin, NF110, NF340 and NF449, lacking one or more of the aromatic rings and accompanying sulfonate groups, had markedly reduced activity (Figure 1 and 5D). Suramin and its analogs might first get close and bind to PAP248-286 via electrostatic interaction. Then, these molecules could adjust themselves to interact with PAP248-286 and stabilize by hydrophobic and hydrogen bonding interactions that involve other parts of the molecule. The net negative charge, the specific orientation of sulfonate groups, the
degree of rigidity and other features of the molecule are key elements for their inhibitory effects.

Currently, there is no approved anti-amyloid treatment that acts as a candidate microbicide. Suramin is a safe medication with nearly a one hundred year-history of clinical use in the treatment of patients with trypanosomiasis. Therefore, suramin displays a major advantage as a result of the relative speed that a candidate microbicide could be developed from the well-recognized knowledge of its pharmacokinetic and safety profiles. Several clinical and preclinical studies have highlighted the pleiotropic beneficial effects of suramin on other clinical domains (8-10,13,27-30). The discovery that suramin could prevent infection by several important viral pathogens, including Zika virus (12), herpes simplex virus (HSV) (31), and others (11,13,27) might indicate that it has potential to be applied to treat multiple sexually transmitted infections.

The success of an effective microbicide requires that particular attention is paid to safety assessment. Despite many years of the systemic administration of suramin in the clinic, topical application remains a safety concern. Suramin showed limited in vitro cytotoxicity to human vaginal epithelial cells, colorectal cells and lymphocytes. Additionally, suramin gel was found to be well tolerated for vaginal use in rabbits. Genital inflammation was associated with increased HIV-1 transmission and decreased potency of ARV drugs (32,33). Suramin has been demonstrated to have potent anti-inflammatory properties (10,29). Our results also showed that suramin gel did not induce inflammation in the vaginal epithelium (Figure 7C-F).

Combination therapy has resulted in the successful treatment of HIV-1. As evidenced by the clinical trial outcomes of polyanions (34), despite possessing multifaceted therapeutic actions, suramin might not be effective enough as a monotherapy to combat various factors during sexual intercourse. In comparison, the synergism of anti-amyloid treatment and antiviral treatment might prove increase treatment performance to stop HIV-1 sexual transmission. Our study also showed that the combination of suramin and ARV drugs resulted in synergism against HIV-1 infection in semen.

SEVI enhances viral infection because it can neutralize the negative charge repulsion that exists between HIV virions and target cells (22). Similarly, SEVI might also neutralize the negative charge repulsion that exists between HIV virions and vaginal epithelial cells, thus promoting the binding and transcytosis of HIV-1 through genital epithelial cells and subsequent infection of target cells (35,36). Our results confirmed this finding that seminal amyloids enhanced viral binding, transcytosis through genital epithelial cells and subsequent infection of target cells (Figure 8). Moreover, suramin could decrease the transcytosis of HIV-1 across genital epithelial cells in the presence of seminal amyloid fibrils and the subsequent infection of lymphocytes.

Our study provided mechanistic insights into the pharmacological actions and the topical safety of suramin, which strongly underscore the hopeful message that suramin should be reconsidered as a
prophylactic supplement and a new combination candidate microbicide to reduce the sexual transmission of HIV-1.

EXPERIMENTAL PROCEDURES

Materials and reagents

Congo red, polybrene were purchased from Sigma (St. Louis, MO). Chemicals, including suramin, NF110, NF157, NF279, NF340, NF449, were bought from Tocris Bioscience. TMC120, AZT, nevirapine, raltegravir and maraviroc were purchased from TargetMol (USA). PROTEOSTAT aggresome detection kit was purchased from Enzo Life Sciences. PAP248-286 and PAP248-286 (Ala) (>95% purity) was synthesized by Scilight-Peptide (Beijing, China). Polyclonal rabbit antibody against PAP248-286 was produced by AbMax Biotechnology Co., Ltd. (Beijing, China). Semen (SE) samples were obtained from healthy lab members with written informed consent in accordance with the Declaration of Helsinki and the study was approved by the Human Ethics Committee of Southern Medical University, China. Ejaculates were liquefied as soon as collected for 30 min at room temperature. Seminal fluid (SE-F), representing the cell free supernatant of SE, was collected by centrifugation of 1 ml SE for 15 min at 10,000 rpm and stored in 1 ml aliquots at -20 °C. Plasmid of EGFP-Vpr, anti-p24 monoclonal antibody (183-12H-5C), were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. Plasmids of various HIV-1 infectious clones were kindly provided by Jan Münch of Ulm University, Ulm, Germany. Viruses were prepared as previously described (6). Cytokine ELISA kits were bought from Dakewe Bioengineering (Shenzhen, China). New Zealand white female rabbits were provided by the Animal Breeding Center of the Southern Medical University (Guangzhou, China). The animal study was approved by the Institutional Animal Care and Use Committee of the Southern Medical University.

Congo red staining assay

Peptide PAP248-286 or PAP248-286(Ala) at 440 μM in PBS was mixed with agents at various concentrations and then agitated at 1400 rpm at 37 °C by using an Eppendorf Thermomixer. Fibril formation was detected as previously described (17).

TEM analysis

Fibrils were generated as described in the Congo red staining section. At indicated time points, aliquots were removed from respective reactions and subjected to TEM analysis as previously described (17).

CD spectroscopy

PAP248-286 at 440 μM was incubated with or without suramin at 880 μM and agitated at 1400 rpm at 37 °C. CD spectra of samples (66 μM) were measured using a J-715 spectrometer (Jasco, Japan) (17).

HIV-1 infection assay

PAP248-286 or whole SE-F was agitated to allow fibril formation with or without suramin as described in the Congo red staining section. Samples at indicated time points were collected and
centrifuged to pellet the fibrils at 12,000 rpm for 5 min to remove the remaining free suramin. The pellets were re-suspended in cultural medium and used to determine their abilities to enhance HIV-1 infection as described previously (6). Briefly, the pellets were mixed with HIV-1 virions for 10 min at room temperature. The virion-fibril mixtures were then used to infect TZM-bl cells (10^4/well). Luciferase activity in triplicate well was measured 48 h postinfection. The enhancement of viral infection was shown relative to those measured in the absence of peptide (\[\text{n - fold enhancement} = \frac{\text{Luciferase(Peptide)} - \text{Luciferase(virus control)}}{\text{Luciferase (virus control)} - \text{Luciferase (cell control)}}\]). The final concentration of PAP248-286 and SE-F in the infection assay was 11 μM and 2.5%, respectively.

To confirm that suramin could diminish the mature fibril-mediated enhancement of viral infection, SEVI or whole SE-F, which has been agitated for 8 h, was incubated with graded concentration of suramin at 37 ºC for 15 min. To eliminate the anti-HIV activity of suramin itself as much as possible, the SEVI or SE-F and suramin mixtures were centrifuged at 12,000 rpm for 5 min to discard the free suramin. Then the pellets were dissolved in fresh medium and were tested their abilities to enhance HIV-1 infection as described above. SEVI was tested at a final concentration of 11 μM, and SE-F was used at a final concentration of 2.5%.

**Tricine-SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot analyses**

PAP248-286 or PAP248-286(Ala) was incubated with suramin at indicated concentration at 37 ºC for 30 min. Mixtures were then centrifuged at 5,000 rpm for 10 min. The free peptide in the supernatant was mixed with loading buffer and boiled at 100 ºC for 10 min. Then the samples were separated by 10-20% gradient tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Peptides were visualized by either Coomassie blue staining or western blotting as indicated elsewhere (17). The primary antibody to recognize PAP248-286 was a polyclonal rabbit antibody against PAP248-286.

**Zeta potential measurement**

SEVI at 220 μM was treated with suramin at indicated concentration. Mixtures were centrifuged for 10 min at 10,000 rpm. The pellets were resuspended in 1 mL of 1 mM KCl. Zeta potential measurements were taken on a Zeta Nanosizer (Malvern, UK).

**Virus pulldown assay**

SEVI fibril at 22 μM was treated with equivoluminal suramin at indicated concentrations at 37 ºC for 30 min. The solutions were centrifuged for 10 min at 5,000 rpm. The pellets were collected and incubated with HIV-1SF162 (20 ng p24) for 30 min at 37 ºC. The samples were centrifuged at 12,000 rpm for 5 min. The proportion of p24 in the pellet was evaluated by ELISA (6).

**Confocal microscopy**

SEVI fibril (220 μM) or whole SE-F was pre-treated with suramin at indicated concentrations at 37 ºC for 30 min. The solutions were centrifuged for 10 min at 5,000 rpm. The pellets were stained with proteostat (6). The mixtures
were centrifuged for 15 min at 5000 rpm, and incubated with EGFP-HIV-1 SF162 for 15 min. Samples were visualized with Zeiss confocal microscope.

**Measurement of the anti-HIV-1 activity of suramin individually and in combination with ARV drugs in semen**

The antiviral activity of different drugs, alone or in combination, was evaluated against HIV-1 SF162 and HIV-1 NL4-3 in TZM-bl cells as previously described (37). Briefly, the pre-cultured TZM-bl cells were infected with HIV-1 at 100 TCID50 (50% tissue culture infective dose) in the presence or absence of an individual inhibitor or two inhibitors in combination at the graded concentration overnight. The culture medium was then changed and luciferase activity was determined 72 h later. The effective 50%/90% inhibitory concentration (IC_{50}/IC_{90}, respectively) and combination index (CI) were calculated using the Compusyn software (37).

**Surface plasmon resonance imaging (SPRI)**

Interactions between the PAP248-286 monomer (100 μM) and each inhibitor at 5 mM were analyzed by the PlexArray HT system (Plexera Bioscience, Beijing, China) at room temperature. Chemicals were immobilized on a sensor chip, and subsequently, PAP248-286 was injected as analytes, using PBS with Tween-20 as a running buffer. For binding studies, PAP248-286 was applied in running buffer at a flow rate of 2 μL/s with a contact time of 300 s and a dissociation time of 300 s. Chip platform was regenerated with gly-HCl (pH 2.0) and washed with the running buffer. All possible binding curves were fitted by Langmuir equation.

**Molecular dynamics (MD)**

MD simulation was performed to investigate the interaction between PAP248-286 and suramin, NF157, and NF449, respectively. The X-ray structure of PAP248-286 (PDB ID: 2L3H) (38) was retrieved from the Protein Data Bank. The structures of suramin, NF157, and NF449 were created and optimized using YASARA (39). The initial binding modes of PAP248-286 and ligands were searched by Autodockvina (40). MD simulations were performed using the GROMACS 4.5.3 (41) software package utilizing the CHARMM27 force field. The system was placed in the center of a cubic box that extended 12 Å from the edge of the complex structure. The TIP3P (42) water molecules were filled with the box with some sodium and chloride ions to offer isotonic condition and neutralize the whole system. Subsequently, the system was energy minimized for 300 steps by releasing all the constraints. Then the resulting minimized systems were used for 100 ns MD simulation. All bond lengths were constrained by LINCS algorithm (43). The electrostatic interaction was calculated by the particle mesh Ewald (PME) method (44). The MD temperature was controlled at 300 K and pressure was set at 1 atm. The MD simulation trajectories were saved in every 2 ps for analysis.

**Cytotoxicity assay and safety evaluation in rabbits**

The potential cytotoxic effects of suramin on various cells were analyzed using XTT assay as described previously (17). The 50% cytotoxicity concentration (CC_{50}) values were calculated. Vaginal irritation studies
were conducted to assess the nonclinical safety of suramin. Vaginal gels were prepared as previously described (45). Sixteen female rabbits in subgroups of four were administered intravaginally with 1 mL of placebo gel, 4% N-9 gel, 1% suramin gel and 4% suramin gel respectively. The placebo gel and N-9 gel were used as positive control group and negative control group, respectively. The rabbits were intravaginally administrated once daily with a 12-cm flexible catheter introducing up to its 8-cm mark for 7 consecutive days. The body weight of rabbits was recorded every day. On day 8, rabbits were killed and their vaginas were surgically excised and medially opened. The vaginal tissues were rapidly removed and parts of the upper, middle, and lower portions of the abdominal vagina were formalin-fixed for histopathology evaluation. In addition, CVLs were collected with 500 μL saline at 24 h intervals before each treatment. CVLs were immediately centrifuged at 1,200 rpm for 10 min at 4 ºC. The supernatants were subjected to determine the levels of proinflammatory cytokines, including IL-6, IL-10, IL-8, and TNF-α using commercial ELISA kits.

**HIV-1 binding assay**

HEC-1A cells were cultured in 24-well-plate to over 95% confluence. SEVI fibril at 10 μM or 2.5% SE-F, which has been agitated for 8 h to allow the formation of new fibrils, and HIV-1SF162 (20 ng p24) were first incubated in the presence or absence of suramin (40 μM) at 37 ºC for 30 min. Then, the samples were centrifuged for 10 min at 5,000 rpm. The virions in the supernatant were transferred to HEC-1A cells. After an 8-hour incubation, the cells were washed with PBS and then lysed to quantify HIV-1 p24 by western blotting.

**HIV-1 transcytosis assay**

To establish an intact genital epithelial layer, HEC-1A cells were plated and grown in a 24-well plate on a transwell polycarbonate membrane containing 0.4 μm pores (Corning). Cells were allowed to grow into a tight, polarized apical and basolateral surfaces. Integrity of epithelial layer was monitored by measuring trans-epithelial electrical resistance (TEER), using a Millicell ERS-2 Volt-Ohm Meter (Millipore), according to the manufacturer’s instructions (46). The monolayers were used only when the TEER was 400 mΩ/cm² or greater. HEC-1A cells were grown to confluence on the upper chamber, while the lower compartment was loaded with TZM-bl cells. After verifying the integrity of the monolayer on the transwell filters, HIV-1 SF162 (30 ng) and SEVI (10 μM) or 2.5% SE-F were added to the apical chamber of the transwell insert in the presence or absence of suramin at different concentrations. After 8 h incubation at 37 ºC, the HEC-1A cells were thoroughly washed with PBS to remove any unbound virus, and then detached and lysed. Then, the amounts of attached or internalized virus were determined by western blotting. TZM-bl cells in the lower chamber continued to grow for another 48 h, and the luciferase activity was determined (47).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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TABLE 1 Combination index and dose reduction values for inhibition of HIV-1 SF162 infection by combining suramin with ARV drugs in semen

| Drug combination, % inhibitory Conc (molar ratio) | CI<sup>a</sup> | Suramin IC<sub>50</sub> (nM)<sup>b</sup> | Dose reduction (Fold) | ARVs IC<sub>50</sub> (nM)<sup>b</sup> | Dose reduction (Fold) |
|-------------------------------------------------|-------------|-----------------|----------------------|-----------------|----------------------|
| Suramin:maraviroc (580:1)                       |             |                 |                      |                 |                      |
| 50                                               | 0.233       | 12587.80        | 361.04               | 3.05            | 0.62                 | 4.90                |
| 90                                               | 0.423       | 21949.60        | 3560.16              | 23.54           | 6.14                 | 3.83                |
| Suramin:AZT (2900:1)                             |             |                 |                      |                 |                      |
| 50                                               | 0.739       | 2840            | 1690                 | 4.13            | 0.58                 | 7.12                |
| 90                                               | 0.332       | 58320           | 16020                | 95.79           | 5.52                 | 17.35               |
| Suramin:Nevirapine (232:1)                       |             |                 |                      |                 |                      |
| 50                                               | 0.653       | 7171.69         | 2946.43              | 52.48           | 12.70                | 4.13                |
| 90                                               | 0.691       | 20206.10        | 11281.60             | 366.68          | 48.63                | 7.54                |
| Suramin:Raltegravir (1812.5:1)                   |             |                 |                      |                 |                      |
| 50                                               | 0.614       | 5205.26         | 2687.58              | 15.14           | 1.48                 | 10.21               |
| 90                                               | 0.447       | 60081.30        | 21715.70             | 139.55          | 11.98                | 11.65               |
| Suramin:TMC120 (5370.4:1)                        |             |                 |                      |                 |                      |
| 50                                               | 0.645       | 4429.52         | 1824.77              | 1.46            | 0.34                 | 4.30                |
| 90                                               | 0.473       | 43038.00        | 8653.02              | 5.92            | 1.61                 | 3.67                |

<sup>a</sup> CI, combination index.

<sup>b</sup> Data are the means of two independent experiments performed in triplicate.
TABLE 2 Combination index and dose reduction values for inhibition of HIV-1 NL4-3 infection by combining suramin with ARVs in semen

| Drug combination, % inhibitory | CI | Suramin Conc (nM)b | ARVs Conc (nM)b | Dose reduction |
|-------------------------------|----|-------------------|-----------------|----------------|
|                               |    | Alone  | Mixture  | Alone | Mixture | Alone | Mixture | Alone | Mixture |
| Suramin:AZT                   |    |        |          |       |         |       |         |       |         |
| (106:1)                       |    | 50     | 0.496    | 102.57| 48.23   | 17.52 | 0.45    | 38.52 |
|                               |    | 90     | 0.184    | 4501.35| 828.79 | 30.50 | 7.82    | 3.90  |
| Suramin:Nevirapine            |    | 50     | 0.646    | 149.10| 72.61   | 25.93 | 4.11    | 6.31  |
| (17.7:1)                      |    | 90     | 0.623    | 745.41| 438.66 | 711.52| 24.83   | 28.66 |
| Suramin:Raltegravir           |    | 50     | 0.704    | 160.67| 60.99  | 28.37 | 9.21    | 3.08  |
| (37.9:1)                      |    | 90     | 0.646    | 952.27| 390.20 | 248.8 | 58.90   | 4.22  |
| Suramin:TMC120                |    | 50     | 0.523    | 160.62| 21.53  | 2.72  | 1.06    | 2.57  |
| (20.4:1)                      |    | 90     | 0.168    | 1566.77| 30.09 | 52.07 | 9.92    | 1.48  | 6.72  |

a CI, combination index.
b Data are the means of two independent experiments performed in triplicate.
TABLE 3 *In vitro* cytotoxicity of suramin\(^a\)

| Cell lines       | Category                                 | CC\(_{50}\) (μM)  |
|------------------|------------------------------------------|------------------|
| VK2/E6E7         | Normal reproductive cells                | >1000            |
| Ect/E6E7         |                                          | >1000            |
| Siha             |                                          | >1000            |
| Hela             |                                          | >1000            |
| HEC-1A           | Reproductive cancer cells                | >1000            |
| HEC-1B           |                                          | >1000            |
| Caco-2           |                                          | >3000            |
| SW620            | Human colorectal carcinoma cell          | 1973.5 ± 454.5   |
| HCF-116          |                                          | 1662.4 ± 229.6   |
| TZM-b1           |                                          | >1000            |
| MT-2             | Lymphocytes                              | >1000            |
| Jurkat           |                                          | >1000            |

\(^a\) Data are the means of two independent experiments performed in triplicate. Data are represented as means ± standard deviations.
TABLE 4 Measurement of cell proliferation in rabbit vaginal tissue after intravaginal application of various gels for 7 days

| Treatment (%) | PCNA-positive nuclei (Mean±SD) |   |   |
|---------------|-------------------------------|---|---|
|               | Epithelium (%)                | Stroma (%) |
| Placebo group | 14.54 ± 11.74                 | 3.72 ± 0.24 |
| N-9group      | 84.49 ± 5.08                  | 27.14 ± 13.15 |
| Suramin group |                               |             |
| 1%            | 21.31 ± 10.88                 | 1.40 ± 0.14 |
| 4%            | 41.96 ± 7.37                  | 2.19 ± 0.47 |
FIGURE LEGENDS

Figure 1. Inhibition of PAP248-286 fibrillization by commercially-available suramin analogs. (A-G) Structures of the tested compounds. (H) PAP248-286 (440 μM) were agitated with 2-fold excess of each suramin-analog. Fibril formation was monitored by Congo red staining at the indicated time points. The data represent means ± SD (n=6) calculated from two independent experiments. Each experiment was performed in triplicate measurements. **, *p<0.01; one-way ANOVA.

Figure 2. Inhibition PAP248-286 aggregation by suramin. (A) PAP248-286 (440 μM) were exposed to various concentration of suramin (220 μM, 880 μM, 3520 μM) at 37°C with constant agitation. Fibril formation was detected by Congo red staining at the indicated time points. Data are presented as means ± SD (n=6). ***, *p<0.001; one-way ANOVA. (B) β-Sheet formation of SEVI fibrils was monitored by CD analysis at the time point of 4 h (left) and 24 h (right). SEVI was tested at 440 μM. The experiment was repeated once and similar result was obtained. (C) Morphological analysis of SEVI fibrils in the presence or absence of suramin (suramin to PAP248-286 molar ration, 2:1) after 4 h (upper panel) or 24 h (lower panel) incubation was visualized by TEM. Scale bars indicate 100 nm. (D-E) Inability of PAP248-286 (D) or SE-F (E) incubated with suramin to enhance HIV-1 infection. PAP248-286 (11 μM) or 2.5% of SE-F with or without suramin was agitated to allow fibril formation. At the indicated time points, the fibrils in the pellet were used to test their abilities to enhance infection of HIV-1 SF162 (left panel), HIV-1 NL4-3 (middle panel) and HIV-1 81A and NL4-3 (right panel) respectively in TZM-bl 48 h later. Shown are mean ± SD means ± SD (n=6) calculated from two independent experiments. Each experiment was performed in triplicate measurements. *, *p<0.05; **p<0.01; ***p<0.001; one-way ANOVA.

Figure 3. Inhibition of SEM1(86-107) fibrillization by suramin. SEM1 (400 μM) were agitated with 10-fold excess of suramin. Fibril formation was monitored by Congo red staining at the indicated time points. The data represent means ± SD of three independent experiments. **p<0.01; one-way ANOVA.

Figure 4. Time courses of PAP248-286 aggregation in the absence or presence of different chemicals. PAP248-286 (440 μM) were agitated with PBS, suramin (20 μM) or cellulose sulfate (20 μM) respectively. The status of peptide aggregation was monitored by Congo red staining at the indicated time points. The data represent means ± SD (n=6) calculated from two independent experiments. Each experiment was performed in triplicate measurements. *, *p<0.05; **p<0.01; ***p<0.001; one-way ANOVA.

Figure 5. Binding of suramin to PAP248-286. (A) PAP248-286 at 110 μM was incubated with suramin at different concentrations in the absence (left panel) or presence (right panel) of polybrene at 37 °C for 30 min. The remaining free PAP248-286 in the supernatants after centrifugation at 5,000 rpm for 10 min was recognized by western blotting (upper panel) or Coomassic blue (lower panel). (B) Inability of suramin to bind to PAP248-286(Ala). PAP248-286(Ala) at 124 μM was incubated with suramin at different concentrations at 37 °C for 30 min. The remaining
free PAP248-286(Ala) in the supernatants was recognized by Coomassic blue. (C) Suramin failed to inhibit PAP248-286(Ala) fibrillization. Data are represented as means ± SD (n=3) of three independent experiments; one-way ANOVA. (D) Different suramin-analogs exhibited distinct abilities to interact with PAP248-286. 110 μM PAP248-286 was incubated with different chemicals (110 μM) at 37 °C for 30 min. The remaining free PAP248-286 in the supernatants was recognized by western blotting (upper panel) or Coomassic blue (lower panel). (E) SPRi determination of the binding affinity of various chemicals toward PAP248-286. The data was representative of triplicate measurements. (F) Representative structures of PAP248-286 bound to suramin, NF157, and NF449 (from left to right) in the upper row, PAP248-286 without inhibitors for clarity in the lower row, revealed by MD simulation. (G) The number of interaction between PAP248-286 with suramin, NF157, and NF449 respectively.

**Figure 6. Blockage of the interaction of SEVI with HIV-1 by suramin.** (A) Zeta potential of SEVI fibril in the presence of suramin. The results are the average values ± SD (n=6) calculated from two independent experiments. Each experiment was performed in triplicate measurements. *, p<0.05; **p<0.01; ***p<0.001; one-way ANOVA. (B) SEVI was incubated with HIV-1 SF162 (20 ng p24) in the presence of different amount of suramin and centrifuged. The amounts of p24 in the pellet were determined by ELISA. Values represents the mean ± SD (n=6) calculated from two independent experiments. Each experiment was performed in triplicate measurements. *, p<0.05, **p<0.01, ***p<0.001 vs SEVI control; one-way ANOVA. (C, E) SEVI (440 μM, C) or SE-F, which has been agitated for 8 h, (E) were pretreated with PBS or suramin (880 μM) for 30 min at 37 °C. Mixtures were stained with Proteostat dye (red) and then incubated with EGFP-HIV-1 (green). Samples were imaged with confocal microscope. Scale bar, 10 μm. (D, F) SEVI (D) or agitated SE-F (F) were incubated with suramin at the indicated concentrations or PBS for 15 min at 37 °C. The mixtures were centrifuged and the pellets were subsequently incubated with HIV-1 SF162 (left panel), HIV-1 NL4-3 (middle panel) and HIV-1 81A and NL4-3 (right panel) respectively. At 48 h after infection, luciferase activity was measured as before. All data give average values ± SD (n=6) calculated from two independent experiments. Each experiment was performed in triplicate measurements. *, p<0.05; **p<0.01; p<0.001 (vs SEVI control in D); one-way ANOVA.

**Figure 7. In vivo safety of intravaginal suramin gel in a rabbit model.** (A). Light-microscopy images of cervicovaginal tissue in groups treated with different gels. On day 7, rabbits were surgically excised, formalin-fixed, and paraffin-embedded by standard histological protocols. Treatment with either 1% or 4% suramin gel resulted in the structure integrity of the vaginal squamous epithelium (SE) and absence of any significant leukocytes influx in the submucosa (SM). In contrast, the N-9-treated tissue showed dramatic vascular changes, leukocyted infiltration and deep epithelial ulceration. Scale bars = 100 μm. (B) Representative PCNA-positive and hematoxylin-counterstained, paraffin-embedded sections of rabbit vaginal tissues treated with indicated gels. Scattered epithelial and stromal cell nuclei stain brown, indicating PCNA expression. Scale bars = 50 μm. (C-F) Cytokine levels, including
IL-6 (C), IL-10 (D), IL-8 (E) and TNF-α (F) in CVLs before and after vaginal application of different gels. Statistical analysis was performed by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001 vs placebo; one-way ANOVA.

Figure 8. Inhibitory activities of suramin on the transcytosis of HIV-1 through genital epithelial cells in the presence of seminal amyloids. (A, B) The p24 protein detection in HEC-1A cells by western blot. Suramin at 40 μM and HIV-1 SF162 (20 ng) were incubated with SEVI (10 μM) (A) or 2.5% SE-F (B) at 37 °C for 30 min. The virions in the supernatant were separated by centrifugation and then transferred to HEC-1A cells. After an 8-hour incubation, the cells were washed and lysed to quantify HIV-1 p24 by western blot. (C) The model of the transwell assay. (D-G) HEC-1A cells were grown on a transwell insert containing 0.4 μm pores while TZM-bl cells were cultured in the lower chamber. HIV-1 (30 ng) and SEVI (10 μM) (D, E) or 2.5% SE-F (F, G) added to the apical chamber in the presence or absence of suramin at different concentration. The bound or internalized HIV-1 were assayed in HEC-1A by western blot after 8 h of incubation at 37 °C (D, F) and luciferase activities of TZM-bl cells were determined after 48 h (E, G). Values are means ± SD of three independent experiments. ***, p<0.001; one-way ANOVA.
FIGURE 1
**FIGURE 2**

A. OD490-650 nm over time for different concentrations of suramin.

B. Emission spectra for HIV-1 SF162 and HIV-1 NL4-3 at 4 and 24 hours.

C. TEM images of virus particles at 4 and 24 hours.

D. Enhancement of infectivity for HIV-1 SF162, HIV-1 NL4-3, and HIV-1 81A and NL4-3.

E. Infectivity (RLU/s x 1000) for different virus samples and treatments.
**FIGURE 3**

SEM1(86-107)

- PBS
- 10 × Suramin

OD490-650 nm vs. Time (h)
FIGURE 4

Graph showing the optical density (OD) at 490-650 nm over time (h) for PAP248-286, PAP248-286 + suramin, and PAP248-286 + cellulose sulfate. The graph indicates a comparison of growth over time with error bars showing variability.
A

Polybrene (2672 μM) - - - - - -
Suramin - 0.25× 0.5× 1× 2×
PAP248-286 (110 μM) + + + + (kDa)

B

Suramin - 0.25× 0.5× 1× 2×
PAP248-286 (Ala) (124 μM) + + + + (kDa)

D

Compound (1×) -
PAP248-286 (110 μM) + + + + + + (kDa)

E

Suramin
K_D=1.18×10^{-6}M

NF157
K_D=3.19×10^{-6}M

NF449
K_D=1.52×10^{-5}M

F

PAP248-286 + Suramin
PAP248-286 + NF157
PAP248-286 + NF449

G

|                  | Suramin | NF157 | NF449 |
|------------------|---------|-------|-------|
| Hydrogen bond    | 21      | 15    | 14    |
| Hydrophobic interaction | 46      | 42    | 27    |
| Electrostatic interaction | 14      | 11    | 10    |
**FIGURE 6**

(A) Enhancement of infectivity (fold) for HIV-1 SF162 with SEVI and suramin. The graph shows the Zeta Potential (mV) for different concentrations of SEVI and suramin. The x-axis represents SEVI (11 µM), suramin, and their combinations, while the y-axis shows the enhanement of infectivity.

(B) Pellet-bound P24% for HIV-1 SF162 with SEVI and suramin. The graph shows the percentage of P24-bound virus for different concentrations of SEVI and suramin. The x-axis represents SEVI (22 µM) and suramin (µM), while the y-axis shows the percentage of P24-bound virus.

(C) Fibrils, HIV-1, and Merge images for SEVI and SEVI + 2×Suramin.

(D) Enhancement of infectivity (fold) for HIV-1 SF162 and HIV-1 NL4-3 with SEVI and suramin. The graph shows the enhancement of infectivity for different concentrations of SEVI and suramin. The x-axis represents SEVI (11 µM) and suramin, while the y-axis shows the enhancement of infectivity.

(E) Fibrils, HIV-1, and Merge images for SE-F and SE-F + Suramin.

(F) Infectivity (RLU/s × 1000) for HIV-1 SF152, HIV-1 NL4-3, and HIV-1 81A and NL4-3 with virus and suramin. The graph shows the infectivity for different concentrations of virus and suramin. The x-axis represents virus (2.5% SE-F) and suramin, while the y-axis shows the infectivity.
FIGURE 7
The anti-parasitic drug suramin potently inhibits formation of seminal amyloid fibrils and their interaction with HIV-1

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