HIV-1 Replication Is Stimulated by Sodium Stibogluconate, the Therapeutic Mainstay in the Treatment of Leishmaniasis

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Leishmaniasis is an important opportunistic disease among patients infected with human immunodeficiency virus (HIV)-1. The pentavalent antimony compound sodium stibogluconate is a drug of choice for the treatment of leishmaniasis. Because sodium stibogluconate acts as an inhibitor of phosphotyrosyl phosphatases and such inhibitors can promote HIV-1 replication, we tested the effect of this compound on virus gene expression. Using pseudotyped reporter viruses and fully infectious laboratory-adapted and clinical strains of HIV-1, we report that sodium stibogluconate induces an increase in HIV-1 transcription and virus replication in primary CD4+ T cells and in thymic histocultures. This activation is a slow process and appears to involve the transcription factors nuclear factor-κB and activator protein 1, as well as the Syk, Jun, and mitogen-activated protein kinase/extracellular signal-related kinase signal-transduction pathways. In addition, the effect seems to be partly mediated by a soluble factor. Altogether, these findings might reveal clinical implications for the treatment of leishmaniasis in HIV-1–infected patients.

Among the various opportunistic pathogens infecting patients with AIDS, the protozoan parasite *Leishmania* causes diseases affecting skin, mucosal surfaces, and/or the visceral reticuloendothelial organs. Given that more than one-third of individuals infected with HIV-1 live in regions where this parasitic infection is endemic (e.g., South Asia, North and East Africa, and southern Europe), visceral leishmaniasis (VL) has emerged as an important opportunistic disease among patients with AIDS. For example, in southern Europe, 25%–70% of adult cases of VL are now associated with HIV-1 [1]. Interestingly, *Leishmania* infection promotes HIV-1 replication in vitro [2, 3] and in vivo [4, 5]. Indeed, VL has been shown to cause an acceleration of the progression of AIDS, mainly through the increased production of proinflammatory cytokines and chronic T cell activation [4, 6, 7]. Moreover, antileishmanial drugs are less efficient and are not well tolerated in HIV-1–infected persons [8–11].

The treatment of choice for leishmaniasis since 1960 has been pentavalent antimony drugs, despite the emergence of large-scale resistance in India [12, 13]. The most commonly used organic compounds of antimony are sodium stiboglucone (Pentostam) and meglumine antimoniate (Glucantime). Apart from its antileishmanial activity, sodium stibogluconate was identified in a screen of chemical reagents as a potential inhibitor of various protein tyrosine phosphatases such as the Src...
homology domain 2–containing tyrosine phosphatase-1 (SHP-1), SHP-2, and protein tyrosine phosphatase 1B [14]. Given the previously reported capacity of sodium stibogluconate to inhibit tyrosine phosphatase activity and that HIV-1 expression is enhanced by treatment with the potent tyrosine phosphatase inhibitors bis-peroxovanadium compounds [15], we measured the effect of antimonial drugs on HIV-1 replication. Our results demonstrate that sodium stibogluconate induces a dose-dependent increase in virus gene expression and replication in primary human CD4+ T lymphocytes and in small blocks of thymic tissue cultured ex vivo. This activation is characterized by a slow kinetics and appears to be partially mediated through the secretion of an undefined soluble factor.

**MATERIALS AND METHODS**

**Cells and tissue culture.** Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors and purified by ficoll-hypaque centrifugation. Human T helper cells (i.e., CD4+ T cells) were negatively isolated from fresh PBMCs using the CD4+ T Cell Isolation Kit (Miltenyi Biotec) in accordance with the manufacturer’s instructions. PBMCs and CD4+ T cells were either used immediately or were activated with phytohemagglutinin-L (1 µg/mL) and interleukin (IL)-2 (30 U/mL) for 48 h. Histocultures of human thymic tissue were prepared as described elsewhere [16].

**Chemicals and antibodies.** The free formulations of sodium stibogluconate (Glaxo-Wellcome; Walter Reed Army Institute of Research [WRAIR] lot reference BL06916) and N-methylglucamine antimoniate (Rhône Poulenc; WRAIR lot reference BL09186) were gifts from Nancy Saravia (Centro Internacional de Entrenamiento e Investigaciones Médicas, Cali, Colombia) and were initially obtained from WRAIR. Potassium antimonyl tartrate hydrate and sodium gluconate were obtained from Sigma Aldrich. The hybridoma cell lines that produce the anti-CD3 OKT3, anti–HLA-DR L243, and anti–intercellular adhesion molecule (ICAM)–1 R6.5 monoclonal antibodies were obtained from Sigma Aldrich. The hybridomas that produce antibodies against the HIV-1 core protein p24 (i.e., 183-H12-5C and 31-90-25) were supplied by the National Institutes of Health (NIH) AIDS Repository Reagent Program and ATCC, respectively.

**Molecular constructs.** The pNL4-3 vector is a full-length infectious molecular clone of HIV-1 (a prototypic X4-tropic variant; NIH AIDS Repository Reagent Program). The luciferase-encoding pNL4-3 Luc′ E′ R′ construct was provided by N. R. Landau (The Salk Institute for Biological Studies, San Diego, CA). The pHCMV-G plasmid expressing the vesicular stomatitis virus envelope glycoprotein G (VSV-G) from the human cytomegalovirus promoter has been described elsewhere [18].

**Production of virus stocks and infection.** Fully infectious virions were generated by transient transfection of 293T cells with pNL4-3, and single-cycle pseudotyped HIV-1 particles were produced by cotransfection of 293T cells with pNL4-3 Luc′ E′ R′ and pHCMV-G. Virus stocks were normalized for virion content using an in-house sensitive double-antibody sandwich ELISA specific for the major core viral p24 protein [19]. The HIV-1 B clade clinical isolates 92HT599 (X4 tropic) and 93BR020 (R5 tropic) (NIH AIDS Repository Reagent Program) were amplified in human PBMCs. Activated PBMCs or purified CD4+ T cells were infected with a fixed amount of virus (5 ng of p24/10^7 cells) for 4 h, washed, and then incubated with sodium stibogluconate at various concentrations for 1–14 days in complete RPMI supplemented with IL-2 (5–10 U/mL). For experiments with inhibitors, cells were treated 24 h after virus infection with the compounds for 60 min and then incubated with sodium stibogluconate for 48 h. The pharmacological agents studied included FK506 (10 ng/mL), piceatannol (10 µmol/L), PD98059 (0.5 µmol/L), RO318220 (1 µmol/L), SC68376 (40 µmol/L), SP600125 (40 µmol/L), U73122 (0.25 µmol/L), and wortmannin (100 nmol/L). For infection of thymic organ cultures, tissue pieces were incubated in medium that contained virus for 3 h at 37°C, washed 3 times, and transferred onto sterile filters placed on top of Gelfoam rafts in 700 µL of Yssel’s medium that contained various amounts of sodium stibogluconate.

**Preparation of nuclear extracts and mobility shift assays.** PBMCs and CD4+ T lymphocytes (10^5) were cultured in complete RPMI supplemented with IL-2 in the absence or presence of sodium stibogluconate (400 µg/mL) for 5 days. Nuclear extracts were then prepared according to a protocol described elsewhere [15]. The following dsDNA oligonucleotides were used as probes and/or competitors: the enhancer region (–107/–77) from the NL4-3 strain of HIV-1 (5′-CAAGGGGACTTTCC-GCTGCGGACCTTTCCAGG-3′), the consensus NF-kB binding site (5′-ATGTAGGGGACTTTCCAGG-3′), the distal nuclear factor of activated T cells (NFAT) binding site from the murine IL-2 promoter (5′-TCGAGCCCGAAGGAAAA-TTGTCTCATG-3′), and the consensus binding site for activator protein (AP)–1 (5′-GGCTTGGATGACATGCGGGA-3′). Mobility shift assays and supershifts were performed as described elsewhere [20].

**Western blot.** Total cell extracts were heated for 5 min at 100°C in 1× sample buffer (62 mmol/L Tris-HCl [pH 6.8], 2% SDS, 5% β-mercaptoethanol, 9% glycerol, and 0.002% bromophenol blue) that contained 1 mmol/L phenylmethylsulfonyl fluoride and was loaded onto SDS–10% polyacrylamide gels. The HIV-1 Gag proteins were detected using an anti-p24 antibody (clone 183-H12-5C), followed by a horseradish peroxidase–coupled anti-mouse antibody. Signals were revealed using...
the enhanced chemiluminescence Western-blotting detection reagent (Amersham).

**Statistical analyses.** The statistical significance of the results was defined by performing 1-way analysis of variance with Dunnet post tests to compare treated versus control samples. All analyses were performed on crude data (i.e., luciferase activity or p24 concentrations). \( P < .05 \) was considered to be significant. InStat software (version 3.05; GraphPad Software) was used for all analyses.

**RESULTS**

*Increased virus expression in primary human cells after sodium stibogluconate treatment.* Treatment of virus-infected PBMCs with sodium stibogluconate induced a dose-dependent increase in HIV-1 long terminal repeat (LTR)–driven luciferase activity, whereas the control molecule lacking antimony (i.e., sodium gluconate) had no such effect (figure 1A). The other commercial formulation of antimony, meglumine antimoniate, was less potent in activating HIV-1 expression. Thus, sodium stibogluconate was used in all subsequent experiments. This compound showed no toxicity for PBMCs at concentrations of up to 1 mg/mL (data not shown). The trivalent form of antimony (i.e., potassium antimonyl tartrate hydrate), which is devoid of any phosphatase inhibitory activity, was very toxic at similar concentrations, in accordance with the high cellular toxicity described for this compound [21]. No effect on HIV-1 expression was observed at subcytotoxic concentrations of this trivalent form (i.e., <10 \( \mu \)g/mL; data not shown). When we performed similar experiments with CD4+ T lymphocytes and macrophages, a significant enhancement of HIV-1 expression was seen in CD4+ T cells (figure 1B), whereas only a weak and variable activation was achieved in macrophages (data not shown). To define whether the observed augmentation in HIV-1–dependent reporter gene activity was paralleled by an enhanced expression of virus-encoded structural proteins, CD4+ T cells were infected with replication-competent NL4-3 particles. Treatment with sodium stibogluconate resulted in a greater expression of the viral Gag proteins (i.e., precursor Pr55\(^{gag}\) and processed p24) as monitored by Western blotting (figure 1C). Interestingly, stimulation of HIV-1 expression by sodium stibogluconate was used in all subsequent experiments.
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Figure 2. Increased virus production in CD4+ T cells and histocultures of lymphoid tissue after sodium stibogluconate (SSG) treatment. A, CD4+ T lymphocytes infected with the clinical isolates 92HT599 (X4 tropic) or 93BR020 (R5 tropic) and treated with SSG at the listed concentrations. Virus production was monitored at day 6 after infection by measuring cell-free p24 contents using a double-antibody sandwich ELISA. Results shown are the means ± SDs of triplicate samples and are representative of 3 independent experiments. B, Histocultures of thymic tissue infected with NL4-3 and treated with SSG at the indicated concentrations. Virus production was monitored at day 14 after infection by assessing the p24 content. Two representative experiments with different donors are shown. Asterisks denote statistically significant data (*; **).
Figure 3. Induction of nuclear translocation of NF-κB and activator protein (AP)–1 by sodium stibogluconate (SSG) treatment. Purified CD4+ T cells and peripheral blood mononuclear cells were either left untreated or treated for 5 days with SSG (400 μg/mL). Nuclear extracts from these cells were used in mobility shift assays using an HIV-1 enhancer probe (A), an NF-κB-specific probe (B), a nuclear factor of activated T cells (NFAT)–specific probe (C), or an AP-1–specific probe (D). In panel A, cells were cultured with 2 U/mL (a) or 20 U/mL (b) of interleukin-2. Competitions were performed with a 100-fold excess of either specific (S) or nonspecific (NS) oligonucleotides. Supershift assays were performed with an anti–NF-κB p50 or p65 antibody. The relative band intensity for treated vs. control samples is indicated at the bottom of individual lanes.

the sodium stibogluconate–mediated induction of HIV-1 transcription (see below). Interestingly, sodium stibogluconate treatment also increased the AP-1 binding activity (figure 3D). However, mobility-shift assays performed with probes specific for cAMP response element binding protein, CCAAT enhanced binding protein, and signal transducer and activator of transcription factor–1/2 and –3 did not reveal any effect of antimony on these transcription factors (data not shown).

To provide additional information about the possible signal transduction pathway(s) engaged after sodium stibogluconate treatment that resulted in the activation of NF-κB and AP-1, experiments were performed with specific chemical inhibitors in CD4+ T cells infected with VSV-G pseudotyped reporter HIV-1 particles. To minimize the degradation of the tested pharmacological agents, target cells were treated with sodium stibogluconate for a short period (48 h). The results of a typical experiment are shown in figure 4A. Because of the high variability observed among the donors, which can be explained by differences in the genetic background as well as the activation status of the various CD4+ T cell preparations, individual data from several experiments are presented in figure 4B. Despite these variations, results show that piceatannol, an inhibitor of Syk-family kinases, as well as the c-Jun N-terminal kinase inhibitor SP600125, were consistently able to partially or totally inhibit activation of HIV-1 gene expression by sodium stibogluconate (figure 4A). A partial reduction was also seen with an inhibitor of MAPK/ERK kinase (PD98059), a kinase upstream of ERK1/2, whereas inhibitors of Src kinases (PP2), PI 3-kinase, protein kinase C (PKC; RO318220), phospholipase C-γ (U73122), and the calcium-calmodulin (FK506) signaling pathway had no reproducible effect (figure 4B).

**Induction by sodium stibogluconate treatment of homotypic**
adhesion of CD4\(^+\) T cells and increase in surface expression of activation markers in CD4\(^+\) T cell blasts. We next tested whether the enhancing effect of sodium stibogluconate on HIV-1 replication could be the consequence of the induction of an activation and/or proliferation cellular state. No induction of proliferation was observed after treatment of quiescent or activated CD4\(^+\) T cells with various concentrations of sodium stibogluconate (data not shown). In addition, incubation of resting CD4\(^+\) T cells with sodium stibogluconate did not modulate the expression of activation markers such as CD69, CD25, and HLA-DR (data not shown). However, a strong homotypic adhesion was observed after such an incubation (figure 5A), and this effect could be related to a modest increase in surface expression of the integrin leukocyte function antigen (LFA)–1 (CD11a/CD18) (data not shown). A similar treatment of activated T cells (blasts) resulted in an increase in CD69, HLA-DR, and ICAM-1 expression levels that was detectable 48 h after the addition of sodium stibogluconate and culminated after 6 days (figure 5B). The increased surface expression of ICAM-1 could be responsible to some extent for the enhanced sodium stibogluconate–mediated homotypic aggregation of CD4\(^+\) T cell blasts (data not shown). LFA-1 expression was very high in such CD4\(^+\) T cell blasts and was not modified by the drug (data not shown).

Secreted factor involvement in sodium stibogluconate–induced HIV-1 LTR activation. The slow kinetics of sodium stibogluconate’s action on HIV-1 gene expression suggests that the compound could act indirectly—for example, through the secretion of some soluble factor(s) that would, in turn, increase HIV-1 expression in an autocrine or paracrine fashion. This hypothesis was tested by preparing conditioned medium from sodium stibogluconate–treated PBMCs. Thereafter, cells were infected with luciferase-encoding VSV-G pseudotypes and were cultured in the conditioned medium. Because the conditioned
Figure 5. Induction of homotypic adhesion and an increased expression of several cell surface molecules in CD4+ T cells by sodium stibogluconate (SSG) treatment. A, Purified resting CD4+ T cells, either left untreated or treated for 24 h with SSG (100 or 500 μg/mL) and phytohemagglutinin-L (1 μg/mL) (used as a positive control). Optic micrographs shown (magnification, ×200) are representative of 5 similar experiments. B, Cell-surface expression of CD69, CD25, HLA-DR, and intercellular adhesion molecule (ICAM)-1 in activated CD4+ T cells left untreated or treated with SSG (400 μg/mL).

medium might contain active residual sodium stibogluconate, the incubation time was limited to 24 h, to avoid any significant direct effect of the drug. The results depicted in figure 6 demonstrate that a more important increase in HIV-1 LTR-dependent reporter gene activity is seen after incubation with conditioned medium, compared with fresh sodium stibogluconate (i.e., a 2.2-fold vs. 1.6-fold increase). In addition, heat treatment of conditioned medium (i.e., for 5 min at 100°C) resulted in a marked diminution of the enhancing effect on HIV-1 transcriptional activity, whereas it had no effect on sodium stibogluconate alone.

DISCUSSION

Our data demonstrate that one of the most widely used anti-leishmanial drugs, sodium stibogluconate, can promote HIV-1 replication. Indeed, we provide evidence that HIV-1 gene expression and virus production are both augmented after the in vitro treatment of purified CD4+ T cells and PBMCs with this compound. Furthermore, a similar up-regulatory effect of sodium stibogluconate on virus production was seen in histocultures of human lymphoid tissue, which represent a microenvironment that approximates very well the conditions prevailing under physiological situations. The mechanism through which this increased HIV-1 expression is achieved is still ill defined. Given that the pentavalent, but not the trivalent, form of antimony displays such an effect, it can be proposed that the up-regulatory effect is related to its previously demonstrated capacity to inhibit tyrosine phosphatase activity [14]. The cellular transcription factors responsible for the induction of virus production are most likely NF-κB and AP-1, with no implication of NFAT. Surprisingly enough, no change in these transcription factors could be detected during the first 48 h of treatment, and this slow kinetics was correlated with a very progressive activation of HIV-1 that culminated after 5 days of treatment in CD4+ T cells and even after 14 days in thymic histocultures.

Although sodium stibogluconate treatment is not sufficient per se to activate quiescent CD4+ T cells, it can increase the expression of ICAM-1 and other activation markers (i.e., CD69 and HLA-DR) in CD4+ T cell blasts. The sodium stibogluco-
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Figure 6. Involvement of soluble factor in sodium stibogluconate (SSG)–mediated activation of HIV-1 gene expression. Conditioned medium was first prepared from peripheral blood mononuclear cells (PBMCs) treated with SSG for 5 days. Next, PBMCs were infected with reporter virus pseudotyped with vesicular stomatitis virus envelope glycoprotein G and incubated with conditioned medium or an equivalent concentration of SSG for 24 h before the assessment of luciferase activity. Where indicated, the conditioned medium was heated for 5 min at 100°C before it was added to the cells. Results are presented as fold induction in luciferase activity of SSG-treated over untreated samples. Asterisks denote statistically significant data between samples treated with conditioned medium and fresh SSG (250 µg/mL) (**P < .01).

nate–mediated induction of ICAM-1 is perfectly in line with our previous observations indicating that the expression of this adhesion molecule is regulated by tyrosine phosphatase activity through the nuclear translocation of different transcription factors, including NF-κB [22]. The increase in CD69 expression could also be related to the activation of NF-κB and to the AP-1 transcription factor [23]. On the basis of this information, it is possible to propose intracellular signalling pathways that could be implicated in the sodium stibogluconate–mediated induction of virus expression. For example, a possible involvement of the MAPK/ERK cascade is suggested by the partial inhibition induced by the ERK inhibitor PD98059 and by the observed activation of AP-1, a transcription factor known to be activated through this pathway. The strong inhibition of activation seen in the presence of the Jun N-terminal kinase (JNK) inhibitor SP600125 suggests the contribution of the SAPK/JNK pathway. Interestingly, both the ERK and JNK pathways are negatively regulated by the tyrosine phosphatase SHP-1 in T cells [24, 25]. Because sodium stibogluconate treatment leads to NF-κB translocation and is inhibited by the Syk kinase inhibitor piceatannol, it could also act through a deregulation of the PI 3-kinase pathway. Interestingly, a recent study described an activation of phosphoinositol 3-kinase, PKC, MAPK P38, ERK-1, and ERK-2 after sodium stibogluconate treatment of Leishmania donovani–infected mouse macrophages [26].

The very progressive up-regulation of HIV-1 expression that is observed after sodium stibogluconate treatment could result from 2 distinct but not mutually exclusive phenomena. First, the sodium stibogluconate–induced phosphatase inhibition could lead to a very weak increase in tyrosine phosphorylation of key signaling molecules and, hence, to a slow accumulation in the nucleus of the transcription factors necessary to drive HIV-1 LTR transcription. Indeed, Western-blot analysis of sodium stibogluconate–treated CD4+ T cells with the phosphotyrosine-specific antibody 4G10 revealed a very weak and transient increase in tyrosine-phosphorylated proteins (data not shown), which is in agreement with observations made in myeloid cell lines [14, 27, 28]. Second, sodium stibogluconate could act indirectly, through the secretion of a soluble factor that would, in turn, activate HIV-1 expression in an autocrine and/or paracrine fashion. Our experiments using sodium stibogluconate–conditioned medium favored the involvement of an as-yet-unidentified heat-labile, secreted factor in HIV-1 up-regulation. Because the increase in expression was gradual rather than delayed, this second mechanism was certainly not sufficient to account for the effects of the drug on HIV-1 replication. The cytokines known to activate HIV-1 expression in T cells include IL-1α/β, IL-2, IL-7, IL-12, IL-15, IL-18, and tumor necrosis factor (TNF–α) [29]. Additional studies to identify the factor involved in this indirect effect are in progress.

The observed enhanced homotypic adhesion of CD4+ T cells after sodium stibogluconate treatment could also explain some of the inductive effects on HIV-1 replication, in that it might increase cell-to-cell transmission. However, this effect is probably limited, because the strongest effect on HIV-1 expression was observed when VSV-G–pseudotyped HIV-1 particles were used, and they can achieve only a single cycle of infection. More likely, the increased homotypic adhesion may lead to more pronounced HIV-1–induced syncytium formation—an event that has been shown to be dependent on adhesion mol-
ecules [30, 31]. This could explain the extended cytopathic effects observed at high concentrations of sodium stibogluconate in cells infected with syncytia-inducing HIV-1 isolates such as 92HTS99 and lead to a diminution in viral production (see figure 2A). Hence, sodium stibogluconate treatment could result in an increase in syncytia-induced CD4+ T cell depletion in vivo.

We previously reported that *Leishmania* infection induced an increase in HIV-1 production in macrophages and human lymphoid tissue through secretion of the proinflammatory cytokines TNF-α and IL-1α [2, 3]—data that parallel clinical findings showing increased viral loads and an aberrant activation of the TNF system in dually infected patients [4, 5]. Therefore, these dually infected patients should be treated with effective antiparasitic agents, to avoid a possible *Leishmania*-mediated amplification of HIV-1 production and subsequent deterioration of the immunological response. Unfortunately, we have demonstrated here that one of the most commonly used compounds for the treatment of leishmaniasis, the antimonial drug sodium stibogluconate (Pentostam), can enhance HIV-1 replication in CD4+ T lymphocytes and lymphoid tissue, which are recognized as natural reservoirs of this pathogen. Pharmacokinetics studies have shown that a blood peak concentration of 10 μg/mL of antimony has been reached after an intramuscular dose of Pentostam at 10 mg/kg of body weight [32, 33]. At the current recommended regimen of 20 mg of antimony/kg of body weight [34], we can assume that a blood concentration of 20 μg/mL is generally reached in treated patients. The sodium stibogluconate compound used for study contains 30% total antimony, as determined by polarography, chromous ion titration, and atomic absorption (N. Saravia, Centro Internacional de Entrenamiento e Investigaciones Médicas, Cali, Colombia, personal communication). Hence, a total antimony concentration of 20 μg/mL corresponds to 60 μg/mL of Pentostam. Our data demonstrate that the stimulation of HIV-1 replication is induced at such a concentration. In addition, although most of the antimony is rapidly eliminated in the blood [32], the drug can accumulate in cells, reaching higher intracellular concentrations [21]. These observations add functional significance to our findings and raise concerns about the current treatments offered to persons coinfected with *Leishmania* and HIV-1. In fact, several reports have shown a high frequency of relapse after antimony treatment [8, 9, 35] and a poor tolerance of this drug [35, 36] in coinfected patients. In view of our results, the mortality among HIV-infected patients that was caused by the sodium stibogluconate treatment itself [35] could be related in part to an acceleration of HIV-1 disease. The clinical data and the data described in the present work suggest that the use of sodium stibogluconate to control parasitemia in patients dually infected with *Leishmania* and HIV-1 should be undertaken with caution.

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References