

Procaine Hydrochloride (SP-01 and SP-01A): *In Vitro* Antiretroviral Properties and Reduction of Viral Load in Patients Using Highly Active Antiretroviral Therapy

Stephen J. Brown¹, Laurent Lecanu^{2,3}, George Fareed¹, Sergio Codina¹, Stanley P. Azen⁴, Wenguo Yao^{2,3}, Janet Greeson⁵, Vassilios Papadopoulos^{1,2}

¹AIDS Research Alliance, West Hollywood, California 90069; ²Department of Biochemistry and Molecular Biology; ³Samaritan Research Laboratories, Georgetown University Medical Center, Washington DC, 20057; ⁴Statistical Consultation and Research Center, Keck School of Medicine, University of Southern California, California 90033; ⁵Samaritan Pharmaceuticals, Las Vegas, Nevada 89109 USA



Objectives: To assess the ability of procaine hydrochloride (SP-01) and its oral formulation SP-01A to block HIV-1 infectivity *in vitro* and to assess the effect of SP-01A in a Phase I/II clinical trial, used in conjunction with stable antiretroviral regimen, on viral load, Whalen Symptom Index, CD4+ and CD8+ lymphocyte subsets, and 24 hour cortisol secretion in HIV-1 infected antiretroviral therapy (ART) experienced patients. **Results:** SP-01A was more potent than the reference compound AZT in reducing viral replication with an IC50 of 2 nM compared to 27 nM for AZT. The IC50 value obtained was highly dependent on the duration of the SP-01A pre-incubation with the cells. Twelve and 24 hours pre-medication resulted in IC50s equal to 132 and 8 nM, respectively. SP-01A also dramatically reduced the multi-drug resistant viral strain MDR-769 replication with an IC50 of 0.6 nM and a maximum inhibitory effect above 75%. SP-01A did not show any toxicity on human macrophages and lymphocytes, neuronal and HeLa cells. In the Phase I/II clinical trial, significant reductions in viral load, up to 1.6 log₁₀, and Whalen Symptom Index; changed values returned to baseline after the study drug was withdrawn were observed. No significant effect of SP-01A on CD3+, CD4+, or CD8+ cell counts was observed. No significant trends in adverse events or clinical laboratory values were observed.

Introduction

Currently approved therapies for HIV-1 target either the viral reverse transcriptase (RT), as in the case of the nucleoside and the non-nucleoside analogs, or the viral protease inhibitors (PI). A triple regimen using a combination of these agents is considered the standard of care and, when effective, results in suppression of the virus below the detection limits. However, the rapid rate of mutation of HIV-1 and conferred resistance of the virus to current therapies continues to necessitate a need for additional therapeutic agents. Several observations have established that inhibitors of cholesterol synthesis inhibit cell fusion formation induced by HIV-1 and that drugs extracting cholesterol from the cellular membrane exert an anti-HIV-1 effect *in vitro*. We previously reported that procaine, the active component of SP-01A, inhibit the hormones-induced steroid synthesis by decreasing *in vitro* the expression of the cholesterol synthesis key enzyme HMG-CoA reductase mRNA. We report herein the results of a clinical phase I/II assessing the effect of SP-01A on the viral load, the Whalen Symptom Index and related factors and the *in vitro* anti-viral effect of SP-01A on the replication of HIV-1 IIB and MDR769.

Material and methods

Part 1. Procaine hydrochloride (SP-01) and its oral formulation SP-01A inhibition of HIV-1IIB and MDR769 infectivity and viral replication *in vitro* using the GenPhar AV-Finder™ HIV Drug Discovery Assay and immunocytochemistry. **Infectivity Setting:** Detector plates are set up at day 1 by adding HeLa cells (3000/well) to the adenovirus AD-3R in DMEM containing CCS in 96-well plates and incubated at 37°C under 95% humidity and 5% CO₂ for 2 days. At Day 3, increasing concentrations of SP-01A or reference compound (AZT) were added and incubated 6, 12, 24 or 48 hours. Thereafter, the medium was replaced by fresh medium containing the corresponding concentration of the compounds of interest and HIV-1 IIB (200IP/well) and the cells were incubated overnight. Then, the medium was replaced by fresh medium containing the corresponding concentration of compounds of interest and the infectivity was assessed by measuring the fluorescence on each well 3 days later (λ_{em}=485 nm; λ_{exc}=520 nm). Results are expressed as percentage of inhibition of the viral replication. The effect of SP-01A on the multi-drugs resistant strain MDR-769 has been assessed without any pre-medication. **Immunocytochemistry Setting:** Engineered HeLa cells were seeded in Lab-Tek II chamber (6000 cells/chamber) and pre-medicated for 48 hours with SP01 at 1 nM. Control cells received standard culture medium. Cells were incubated at 37°C under 5% CO₂ and 95% humidity. The HIV-1 IIB infectious viral particles (IVP) were added at the end of the pre-medication period (500 IVP/well). The infection reaction was stopped by adding 4% PFA fixative buffer to the well at 105 minutes.

Part 2. The design for the clinical trial was a Phase I/II non-randomized, open-label, single center, eight-week study using four doses of orally administered Procaine HCl: 200 mg (Cohort A), 400 mg (Cohort B), 600 mg (Cohort C) and 800 mg (Cohort D). **Setting:** The clinical trial was an outpatient, open-label study at one investigative center conducted at AIDS Research Alliance, West Hollywood, California. **Patients:** Twenty-nine (29) male HIV-1 positive, ART-experienced patients entered the study treatment phase (Cohorts A, B, C, and D). Non HIV-infected patients were also enrolled in Cohort E as controls for cortisol secretion; these patients did not receive treatment. Of the 29 patients enrolled in the study treatment phase, 9 were Caucasian (31%), 6 were Hispanic (21%), 9 were African American (31%), and one was self-defined as "Other" (3%). Ages ranged from 32 to 61 years. **Intervention:** Patients were received one of four doses of orally administered Procaine HCl: 200 mg (Cohort A), 400 mg (Cohort B), 600 mg (Cohort C) and 800 mg (Cohort D) and were treated for 8 weeks.

Fig. 1: Inhibitory effect of SP-01A pre-medication on the HIV-1 IIB strain replication

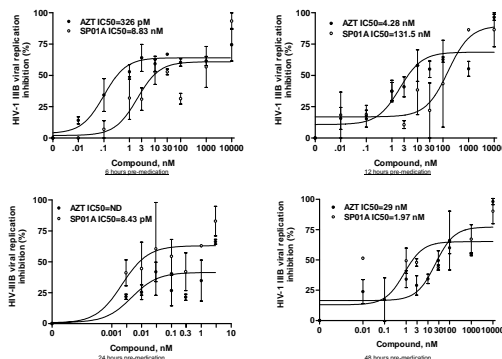


Fig. 2: Inhibitory effect of SP-01A pre-medication on the HIV-1 MDR769 strain replication

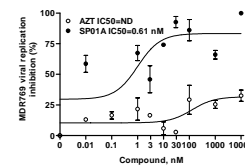


Fig. 3: Effect of SP-01 (1 nM) pre-medication on HIV-1 IIB infectivity studied on engineered HeLa cells expressing CD4, CXCR4 and CCR5. The HeLa cells have been incubated with SP-01A for 48h and infected with HIV-1 IIB for 105 min.

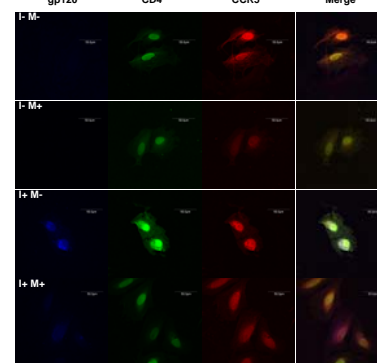


Table 1: Whalen Symptom Index

Index Score	Symptoms
0	No adverse health symptoms
0-5	Some adverse health symptoms
6-10	Intermittent bouts with severe adverse health symptoms
11-15	Regular bouts with severe adverse health symptoms
16-20	Numerous bouts with severe adverse health symptoms

Fig. 3 Whalen Symptom Index Scores

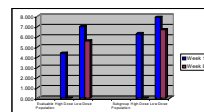


Fig. 4 Proportion of subjects attaining HIV-1 RNA LDL (Weeks 1 – 8)

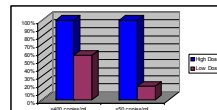


Fig. 6 Proportion of Subjects with >1.0 log₁₀ Decrease (Weeks 1 – 8)

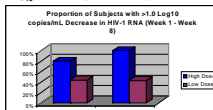


Fig. 5 Log₁₀ HIV-1 RNA Viral Load Reduction (Weeks 1 – 8)

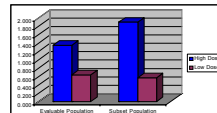


Table 2: Adverse Events Table by Subjects

Adverse Event**	All Subjects (N=29)	High Dose (N=12)	Low Dose (N=17)
Increased ALT*	2 (7%)	1 (8%)	1 (6%)
Increased Amylase	2 (7%)	1 (8%)	1 (6%)
Increased AST	2 (7%)	1 (8%)	1 (6%)
Increased Bilirubin*	2 (7%)	0	2 (12%)
Bronchitis*	2 (7%)	1 (8%)	1 (6%)
Increased Cholesterol*	2 (7%)	0	2 (12%)
Cough	2 (7%)	1 (8%)	1 (6%)
Diarrhea	2 (7%)	0	2 (12%)
Fatigue	2 (7%)	0	2 (12%)
Fever*	4 (14%)	3(25%)	1 (6%)
Increased Glucose	3 (10%)	0	3 (18%)
Headache	4 (14%)	2 (17%)	2 (12%)
Insomnia	2 (7%)	2 (17%)	0
Nausea	4 (14%)	2 (17%)	2 (12%)
Nervous	2 (7%)	1 (8%)	1 (6%)
Increased SGOT	3 (10%)	2 (17%)	1 (6%)
Skin Rash*	2 (7%)	1 (8%)	1 (6%)
Decreased Sodium*	2 (7%)	0	2 (12%)
Sore Throat	2 (7%)	1 (8%)	1 (6%)
Swollen Turbinates	3 (10%)	1 (8%)	2 (12%)
URI	2 (7%)	1 (8%)	1 (6%)

*At least one adverse event reported during the post-treatment phase of the study.
**Multiple occurrences of the same adverse event in one subject counted only once.

Conclusions: Preliminary data suggest that the effect of SP-01A may be mediated by changes at the cholesterol-protein ratio and interaction at the plasma membrane leading to reduced entry of the virus. This hypothesis is supported by immunocytochemical studies showing reduced presence of gp120 inside transfected HeLa cells pre-medicated with SP-01A before incubation with HIV-1IIB virus. The fact that SP-01A was more active after 48 hours pre-incubation time whereas procaine is metabolized within minutes suggests that its mechanism might involve a metabolic effect rather than a direct effect on the virus. These data suggest that SP-01A may offer the first oral medication blocking HIV infectivity through a novel mechanism. The Phase I/II clinical study enrolled patients who were receiving stable antiretroviral treatment upon entry into the study and who had, in general, adequate control of their HIV-1 infection at baseline (<5000 cells/mL). In spite of this, the patients in the high dose group still achieved a mean decrease of >1.0 log₁₀ copies/mL in HIV-1 RNA without compromising their safety. The positive efficacy changes seen in this study taken together with SP-01A's favorable safety profile clearly indicate an effect that warrants further examination of the higher doses of SP-01A in future studies. In addition, SP-01A did not display any toxicity *in vitro* and very low side-effects during the clinical trial.