The Chinese herb-derived Sparstolonin B suppresses HIV-1 transcription

Xin Deng, Yaping Zhang, Feng Jiang, Ran Chen, Peichun Peng, Bin Wen and Jian Liang*

Abstract

Background: The Chinese herb derived Sparstolonin B, (SsnB), is a recently identified natural compound that selectively blocks TLR2- and TLR4-mediated inflammatory signaling. But it is unknown whether this compound has any effect on HIV infection.

Findings: We found that SsnB treatment blocked HIV-1 transcription via a novel mechanism that requires the TAR region. Treatment of human T cell lines or peripheral blood mononuclear cells with SsnB at 1 μM significantly inhibited HIV production. Lastly, SsnB was able to inhibit HIV in synergy with AZT.

Conclusions: These data suggest that SsnB is a novel natural compound that inhibits HIV-1 transcription and may be a new drug in the treatment of HIV infection.

Keywords: Sparstolonin B, HIV transcription, TAR region

Findings

Despite the success of highly active antiretroviral therapy (HAART) in containing human immunodeficiency virus (HIV) infection, there has been an urgent demand for cheaper and alternative drugs in developing countries. Moreover, HIV persists in stable reservoirs harboring chromosomally integrated latent HIV-1 proviruses, where continuous viral production and reactivation of transcription from these reservoirs are not affected by current drugs [1–4]. As such, novel classes of antivirals are needed to inhibit these processes. In this regard, a drug that blocks HIV transcription would be of great value because it offers the potential to shut down the transcription in HIV latent reservoirs.

SsnB was isolated from a Chinese herb, Spaganium stoloniferum [5, 6] and was recently reported to block TLR2 and TLR4 pathways [7]. Here we report that SsnB is a potent inhibitor of HIV infection. Specifically, we performed a dose response experiment by treating HIV pNL4.3 infected CEM-SS cells with various doses of SsnB. Forty eight hours post-infection we harvested supernatants and titrated the infectivity on the indicator cell line TZM-bl [8]. It was observed that SsnB treatment reduced the infectivity of the supernatants by more than 10 fold (Fig. 1a). To expand our observation, we repeated the experiment using peripheral blood mononuclear cells (PBMCs). Similarly, increasing concentrations of SsnB inhibited the production of HIV, measured by the release of p24 into the supernatants (Fig. 1b).

Next, we sought to determine if SsnB inhibits HIV-1 transcription. To this end, we performed luciferase reporter assays. 293T cells were transfected by HIV-1 LTR-driven luciferase reporter constructs and then treated by SsnB at various concentrations. HEK293T cells in 24-well plates were transfected with 0.2 μg reporter plasmid. 0.05 μg pGL4.74[hRluc/TK] was included to control for transfection efficiency. Dual luciferase assay was performed. In support of our findings, SsnB treatment indeed inhibited the luciferase activity (Fig. 2a). Of note, HIV-1 LTR transcriptional activity was significantly increased upon phorbol myristate acetate (PMA) stimulation, whereas SsnB treatment reduced it by nearly 30 fold (Fig. 2a). To ensure that the observed effect was not due to the cytotoxicity of SsnB, we sought to determine the CC50 of the compound and found no cytotoxicity even at the highest concentration that was tested (Fig. 2b).
To investigate the potential mechanism of inhibition, we generated successive deletion constructs by removing the two NF-κB binding sites, three Sp1 binding sites, and the TAR region from HIV-1 LTR [9, 10] (Fig. 3a). All of these LTR fragments were synthesized at WuXi AppTec (China) and subcloned into the pGL4.11[luc2P] plasmid (Promega). We transfected 293T cells with these constructs and treated cells with SsnB. While the removal of the NF-κB or Sp1 binding sites has no effect on SsnB-mediated inhibition, the ΔTAR construct became non-responsive to SsnB treatment (Fig. 3b). To corroborate this finding, we co-transfected a minimal LTR-Luc construct containing the TAR region with a Tat expressing plasmid into 293T cells. In the presence of SsnB, Tat-induced LTR transcription was severely inhibited (Fig. 3c). Altogether, our results suggest that SsnB is inhibiting HIV transcription via a novel mechanism that requires the presence of TAR region. Of note, the ΔTAR construct remained transcriptionally active, albeit at much lower efficiency, which is consistent with what has been reported recently [11]. The TAR region is very important for HIV Tat-dependent transcriptional activation [12–14]. It would be interesting to test in the future whether SsnB exerts its inhibitory effect by directly interacting with TAR region or with TAR-region binding proteins such as HIV Tat or its cofactor. Further effort to identify cellular or viral targets of SsnB would be crucial in understanding the mechanism of SsnB-mediated blockage of HIV transcription.

Lastly, we tested whether SsnB is able to act synergistically with approved antivirals. To this end, we tested...
the IC$_{50}$ of SsnB with the chain terminator of HIV replication, azidothymidine (AZT) [15]. Data from the drug combination experiments were analyzed according to theorem of Chou-Talalay [16, 17]. In each experiment, the dose-effect data for each single agent and the combination were used to plot a median-effect curve [16, 17]. The derived curves are used for calculation of the combination index (CI) (Table 1) as described previously [18]. The resulting combination index (CI) offers quantitative definition for additive effect (CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combinations [16, 17]. Shown in Table 1, SsnB displayed synergy with AZT when administered together. Altogether, our findings show that SsnB is a novel natural compound that exerts anti-HIV activity by suppressing HIV transcription through the TAR region. These exciting results warrant future studies in testing its effect in combination with other known HIV drugs. It is possible that SsnB may become a new class of anti-HIV drugs that is more affordable.

### Table 1: Synergism between SsnB and AZT

| Expt no. | Concentration of: | CI at HIV-1 inhibition of: |
|----------|-------------------|---------------------------|
|          | AZT (μM)          | SsnB (μg/ml)              |
|          |                   | 50% | 75% | 90% | 95% |
| 1        | 0.04, 0.16, 0.64, 1.28 | 0.1, 0.5, 1, 10 | 0.87 | 0.63 | 0.47 | 0.38 |
| 2        | 0.0025, 0.005, 0.01, 0.02 | 0.5, 1, 10, 50 | 0.77 | 0.53 | 0.46 | 0.43 |

0.5 $\times$ 10$^6$/ml CEM-SS cells were exposed to pNL4.3 virus (p24 = 100 ng) as inoculum in flasks containing either single agent (four concentrations each) or four combinations of AZT and SsnB (for example, in Exp 1, 0.04 μM AZT + 0.16 μM SsnB; 0.16 μM AZT + 0.5 μg/ml SsnB, and so on) for 12 h. Forty eight hours thereafter, the production of infectious virus in the supernatants was determined by the standard TZM-bl assay [8]. The calculation was done using the method described in [18, 19]. CIs of < 1, 1, and >1 indicate synergism, additive effects, and antagonism, respectively.

### Competing interests

The authors declare that they have no competing interests.

### Authors’ contributions

JL and XD designed the overall experiments; XD, YZ, FJ, RC, PP, BW performed the experiments and analyzed the data; XD and JL wrote the manuscript. All authors read and approved the final manuscript.

### Acknowledgments

This study was sponsored by National Natural Science Foundation of China Grant 121704580, The Ministry of Education of the People’s Republic of China grant NCET-13-0745, Guangxi Science and Technology key projects 1298003-1-1, 1355006-7, and 14124004-2-2. J. L. is a Guangxi Bagui Scholar.

Received: 18 February 2015 Accepted: 3 July 2015

Published online: 25 July 2015

### References

1. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen IS. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell. 1990;61(2):213–22.
2. Bukrinsky MI, Stanwick TL, Dempsey MP, Stevenson M. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. Science. 1991;254(5030):423–7.
3. McDougal JS, Mawle A, Cort SP, Nicholson JK, Cross GD, Scheppeler-Campbell JA, et al. Cellular tropism of the human retrovirus HIV-IV/IIA/LAV. I. Role of T cell activation and expression of the T4 antigen. J Immunol. 1985;135(5):3151–62.
4. Ganesh L, Burstein E, Guha-Niyogi A, Louder MK, Mascola JR, Komp LW, et al. The gene product Murr1 restricts HIV-1 replication in resting CD4+ T cells. Nature. 2003;426(6968):853–7. doi:10.1038/nature02171.
5. Qiu C, Xu X. H. Z. Pharmacology and clinics of Chinese material. Medica. 2006;14.
6. Lee SY, Choi SJ, Lee JH, Lee DU, Lee KR. A new phenylpropene glycoside from the rhizome of Sparganium stoloniferum. Arch Pharm Res. 2010;33(4):515–21. doi:10.1007/s12272-010-0040-1.
7. Liang Q, Wu Q, Jiang J, Duan J, Wang C, Smith MD, et al. Characterization of sparstolonin B, a Chinese herb-derived compound, as a selective Toll-like receptor agonist with potent anti-inflammatory properties. J Biol Chem. 2011;286(30):26470–9. doi:10.1074/jbc.M111.227934.
8. Wei X, Decker JM, Liu H, Zhang Z, Aran RB, Kliby JM, et al. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob Agents Chemother. 2002;46(6):1896–905.
9. Garcia JA, Harrich D, Soultanakis E, Wu F, Mitsuyasu R, Gaynor RB. Human immunodeficiency virus type 1 LTR TATA and TAR region sequences required for transcriptional regulation. Embo J. 1989;8(3):765–78.

10. Southgate CD, Green MR. The HIV-1 Tat protein activates transcription from an upstream DNA-binding site: implications for Tat function. Genes Dev. 1991;5(12B):2496–507.

11. Shridhar V, Chen Y, Gupta P. The CDB antiviral factor (CAF) can suppress HIV-1 transcription from the long terminal repeat (LTR) promoter in the absence of elements upstream of the CATATAA box. Virol J. 2014;11:130. doi:10.1186/1743-422X-11-130.

12. Dingwall C, Emberg M, Gait M, Green SM, Heaphy S, Karn J, et al. Human immunodeficiency virus 1 tat protein binds trans-activation-responsive region (TAR) RNA in vitro. Proc Natl Acad Sci U S A. 1989;86(16):6925–9.

13. Weeks KM, Ampe C, Schultz SC, Steitz TA, Crothers DM. Fragments of the HIV-1 Tat protein specifically bind TAR RNA. Science. 1990;249(4974):1281–5.

14. Roy S, Delling U, Chen CH, Rosen CA, Sonenberg N. A bulge structure in HIV-1 TAR RNA is required for Tat binding and Tat-mediated transactivation. Genes Dev. 1990;4(8):1365–73.

15. Broder S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. Antiviral Res. 2010;85(1):1–18. doi:10.1016/j.antiviral.2009.10.002.

16. Chou TC. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res. 2010;70(2):440–6. doi:10.1158/0008-5472.CAN-09-1947.

17. Greco WR, Bravo G, Parsons JC. The search for synergy: a critical review from a response surface perspective. Pharmacol Rev. 1995;47(2):331–85.

18. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul. 1984;22:27–55.

19. Eron Jr JJ, Johnson VA, Merril DP, Chou TC, Hirsch MS. Synergistic inhibition of replication of human immunodeficiency virus type 1, including that of a zidovudine-resistant isolate, by zidovudine and 2',3'-dideoxycytidine in vitro. Antimicrob Agents Chemother. 1992;36(7):1559–62.