

Small molecule inhibitor of HIV-1 nuclear import suppresses HIV-1 replication in human lymphoid tissue ex vivo: a potential addition to current anti-HIV drug repertoire

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Abstract

Despite recent progress in anti-HIV therapy, which has to do mainly with introduction of protease inhibitors into clinical practice, drug toxicity and emergence of drug-resistant isolates during the long-term treatment of the patients necessitates search for new drugs that can be added to currently used components of a multi-drug cocktail in highly active anti-retroviral therapy (HAART). Recently, we described a class of arylene bis(methylketone) compounds that inhibit nuclear import of HIV-1 pre-integration complexes and suppress viral replication in macrophages and PBMC in vitro. In this report, we demonstrate that one of these compounds, CNI-H1194, inhibited HIV-1 replication in primary lymphoid tissue ex vivo. The compound did not antagonize the activity of currently used anti-HIV drugs that inhibit viral reverse transcriptase or protease. These results suggest that arylene bis(methylketone) compounds might be a valuable addition to HAART. © 2000 Elsevier Science B.V. All rights reserved.

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The step of nuclear translocation of the HIV-1 pre-integration complex (PIC) is essential for viral replication as it allows the PIC to get into contact with the cellular chromatin. In non-prolif-

erating cells, such as terminally differentiated macrophages, this process is driven by an interaction between the viral PIC proteins carrying specific nuclear localization signals (NLSs), namely matrix antigen (MA) and integrase (IN), and the cellular nuclear import machinery (for recent reviews see Bukrinsky and Haffar, 1997, 1998; Fouchier and Malim, 1999). In rapidly dividing cells, such as mitogen-stimulated T cells, in addi-

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tion to active nuclear import described above, the viral PIC can get access to the nuclear compartment during mitosis, when the nuclear membrane disassembles. Recently, we described a class of arylene bis(methylketone) compounds that specifically inhibit nuclear translocation of the HIV-1 PIC (Dubrovsky et al., 1995). The lead compound from this group, CNI-H1194 (Fig. 1), demonstrated potent anti-HIV activity in primary macrophage and PBMC cultures (Popov et al., 1996; Haffar et al., 1998) and promising pharmacokinetic characteristics (Berger et al., 1997). We wanted to test the anti-HIV effect of this compound under conditions more closely resembling in vivo situation than those used in tissue culture studies. This was especially important in view of the data indicating that most of the virus detected in the blood of HIV-infected patients comes from T lymphocytes (Perelson et al., 1996, 1997), which might be resistant to the effect of nuclear import inhibitors due to high level of activation and proliferation. CNI-H1194 inhibits HIV-1 replication in lymphoid histocultures.

A recently developed system for culturing human tonsil tissue (Glushakova et al., 1995, 1997) provided an opportunity to test the anti-HIV activity of the compound in the context of intact lymphoid tissue, which is believed to be the main reservoir of replicating HIV-1 in the body of infected individuals (Embretson et al., 1993; Pantaleo et al., 1993a,b). We infected tissue blocks with HIV-1_{SF162}, an R5 isolate that replicates in both primary macrophages and CD4⁺ T

lymphocytes. Results presented in Fig. 2A demonstrate a potent, dose-dependent anti-HIV effect of CNI-H1194 in this system. Although the level of virus replication varied between tissue blocks obtained from three different donors (total p24 production by untreated histocultures during the 14-day experiment was 39.6, 198.4, and 43.6 ng for donors 1, 2, and 3, respectively), the antiviral activity of the compound was rather consistent (Fig. 2B). Importantly, this anti-HIV activity was not associated with cytotoxicity of the compound as demonstrated by flow cytometric analysis of cells shaken out of tissue blocks of CNI-H1194-treated and untreated uninfected lymphohistocultures (Fig. 3). Such analysis truly represents the situation within intact tissue blocks with regard to cell numbers (Grivel and Margolis, 1999). Note that the number of live T cells and the CD4⁺/CD8⁺ ratios in drug-treated and control histocultures (5.53 and 5.47, respectively) were very similar. This is consistent with the notion of quantitative preservation of the main T cell subsets in drug-treated histocultures.

HIV-1 replication in lymphoid tissue occurs predominantly in CD4⁺ T lymphocytes (Glushakova et al., 1999); however, in contrast to PHA-stimulated PBMC cultures, human tonsil system supports productive HIV-1 infection in the absence of exogenous stimulation or activation (Glushakova et al., 1995) and therefore the rate of T cell proliferation in this system is limited. Under such conditions, viral nuclear access depends on active transport of the PIC through the intact nuclear membrane, thus explaining sensitivity to CNI-H1194. This result, together with recent reports demonstrating that non-proliferating, partially activated T cells might represent a residual reservoir of replicating virus in highly active anti-retroviral therapy (HAART)-treated patients (Finzi et al., 1997; Zhang et al., 1999), supports the notion that HIV-1 nuclear import may present an attractive target for anti-HIV therapeutics. CNI-H1194 does not antagonize RT inhibitors.

While the main mechanism of action of arylene bis(methylketone) compounds is inactivation of basic-type NLSs in MA and, presumably, IN through formation of reversible Schiff bases with lysine residues (Dubrovsky et al., 1995), the selec-

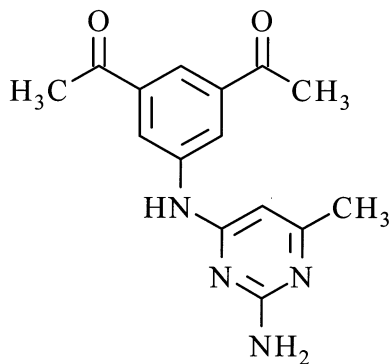


Fig. 1. The chemical structure of CNI-H1194.

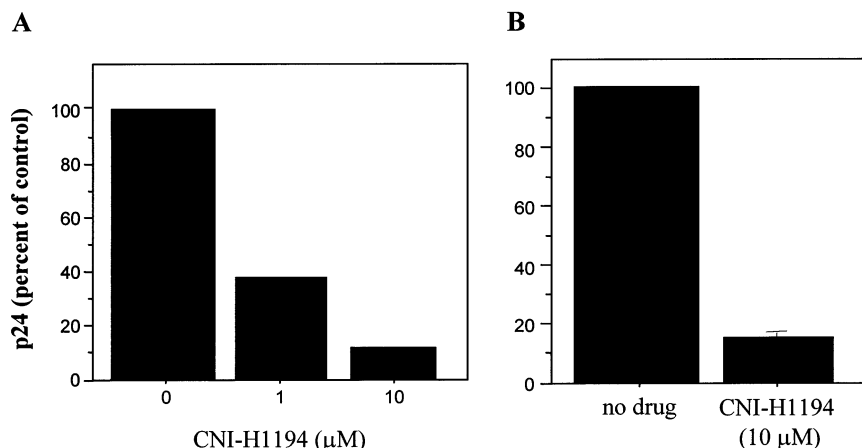


Fig. 2. Anti-HIV activity of CNI-H1194 in histocultures of human tonsils. Human tonsils were obtained from patients undergoing tonsillectomy. Tissues were dissected into 2-mm blocks and incubated on the top of collagen gels at the air–liquid interface (histoculture) as described in detail before (Glushakova et al., 1995, 1997, 1999). The CNI-H1194 drug was added to the medium 3 h prior to infection and was replenished with each medium change. Tissue blocks were infected by slowly applying 3 μ l of clarified virus-containing medium on the top of each tissue block. For each experiment, supernatants from 20 tissue blocks (this number was found empirically to produce statistically reliable data (Glushakova et al., 1995, 1997, 1999)) obtained from the same donor were pooled and used for p24 analysis (measured by ELISA). Panel A: Dose-dependent effect of CNI-H1194 on HIV-1_{SF162} replication in human lymphoid tissue ex vivo. Shown is the amount of p24 antigen (as a percent of p24 in treated versus untreated, control cultures) in the pooled culture medium from 20 tissue blocks from an individual donor, produced over a 14-day incubation. Total amount of p24 produced by control blocks was 43.62 ng. Panel B: The effect of CNI-H1194 (10 μ M) on HIV-1_{SF162} replication in lymphoid tissue from three different donors. The data are presented as a percent of p24 produced by drug-treated tissue blocks relative to non-treated control cultures and are mean \pm S.E.M. of three experiments with tissues from different donors.

tivity for HIV-1-derived NLSs appeared to come from the ability of the compounds to bind to reverse transcriptase (RT) and thus accumulate in the PIC (Popov et al., 1996). This interaction with RT might involve the same site as used by such RT inhibitors as AZT or 3TC, and thus might preclude the use of NLS inhibitors as additions to current formulations of HAART. In our previous study (Dubrovsky et al., 1995), another compound from the same group, CNI-H0293, did not significantly reduce the amount of reverse transcription products produced in an HIV-1-infected cell, suggesting that the compound does not inhibit RT activity. A similar result was obtained with CNI-H1194 (not shown). To measure directly the effect of the compound on HIV-1 RT activity, we added different concentrations of CNI-H1194 to the in vitro RT reaction performed with supernatant of HIV-1-infected culture. As shown in Fig. 4A, no inhibitory activity of the compound was observed at concentrations up to

100 μ M, supporting our previous conclusion that the anti-HIV activity of this class of compounds does not involve RT inhibition (Popov et al., 1996).

To determine whether interaction of CNI-H1194 with RT would diminish the anti-HIV activity of a RT inhibitor, AZT, we measured virus production in the supernatant of HIV-1-infected macrophage cultures treated with different concentrations of AZT in the presence or absence of a 50% inhibitory concentration (100 nM) of CNI-H1194. As shown in Fig. 4B, inhibition of RT activity in the supernatant of cultures treated with a combination of drugs was substantially higher than in cultures treated with AZT alone, indicating that the two compounds do not negatively interfere with one another's activity. Cell viability, as measured by trypan blue staining or using lactate dehydrogenase assay, was approximately 98% in both AZT and AZT + CNI-H1194-treated uninfected cultures, indicating that

the anti-HIV effect was not caused by cytotoxicity of the drug combination. Low cytotoxicity of the compound in macrophage cultures correlated with the results obtained with PBMC (Haffar et al., 1998), and indicated that CNI-H1194 did not alter normal cell nuclear import and metabolism. A

similar result was observed with another RT inhibitor, 3TC (Fig. 4C). CNI-H1194 inhibits replication of AZT-resistant isolate. We also measured the effect of CNI-H1194 on replication of AZT-resistant HIV-1 variant and its parent, pre-drug isolate. Because these viruses replicated poorly in

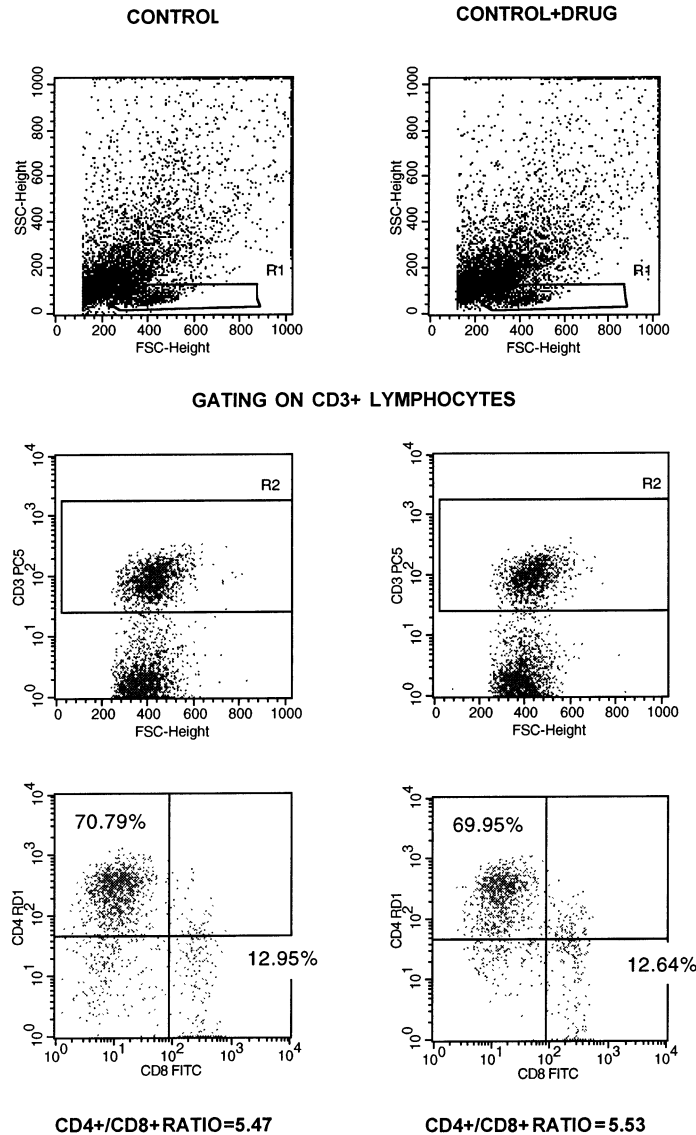


Fig. 3. The effect of CNI-H1194 on T cell subsets in histocultures of human tonsils. Histocultures were prepared as described in the legend to Fig. 2. Two sets of 20 tissue blocks each were cultured with or without addition of 10 μ M of CNI-H1194. Cells were mechanically isolated from tissue blocks on day 15 of culture and stained with a mixture of anti-CD3 (labeled with PC5), anti-CD4 (labeled with RD1), and anti-CD8 (labeled with FITC) monoclonal antibodies (Coulter Corp., Miami, FL). After gating on live CD3⁺ cells (panels in the top two rows), CD4⁺ to CD8⁺ ratio was calculated from the data presented in the bottom row panels.

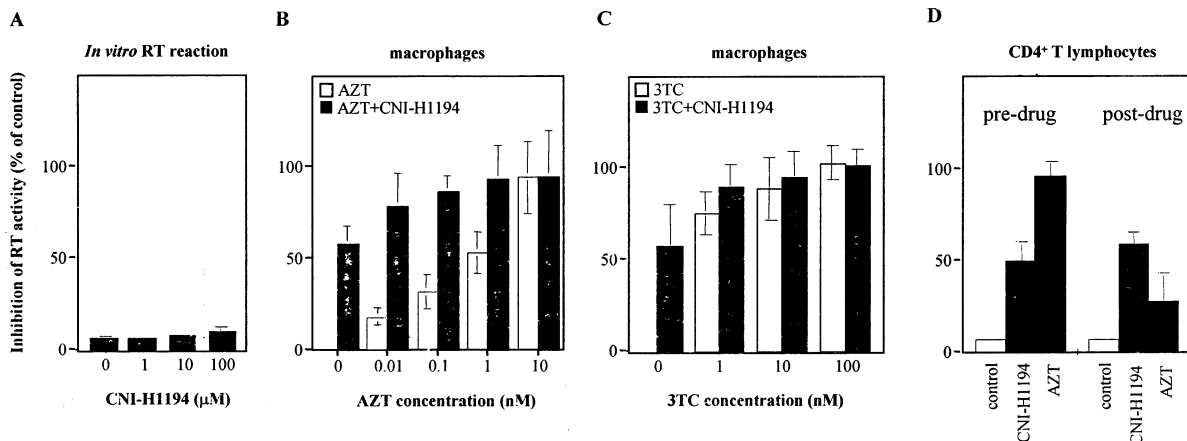


Fig. 4. Anti-HIV activity of CNI-H1194 combination with reverse transcriptase inhibitors. Panel A: Indicated concentrations of CNI-H1194 were added to in vitro reverse transcription reaction performed with a supernatant of HIV-infected culture. Results show mean of three measurements \pm S.D. Panels B, C: Cultures of primary macrophages were prepared from peripheral blood of HIV-seronegative donors as described previously (Schmidtayerova et al., 1997) and infected with a macrophage-tropic HIV-1 strain ADA. Cells were cultured without addition of the drugs or in the presence of indicated concentrations of reverse transcriptase inhibitors AZT (panel A) or 3TC (panel B) with or without 100 nM of CNI-H1194. RT activity in culture supernatants was assayed at various intervals after infection. Results are presented as a reduction of RT activity in treated versus untreated (control) cultures on day 11 after infection. Similar numbers were obtained at other time points. The data are presented as mean of three independent wells, and error bars show standard error. Panel D: PHA-activated CD8⁺ T cell-depleted PBMC were incubated for 10 days in IL-2 and infected with AZT-resistant virus A012 G691-2 (post-drug) or its parental isolate A012 G762-3 (pre-drug) in the absence (control) or presence of 1 μ M of AZT or 10 μ M of CNI-H1194. RT activity in triplicate culture supernatants was evaluated on day 10 after infection and is presented as a mean reduction of RT activity in treated versus control cultures \pm S.D.

macrophage cultures, the analysis was performed in CD4⁺ T lymphocytes. To allow these cells to return to a semi-quiescent state after in vitro activation and thus to partially avoid the negative effect of cell proliferation on CNI-H1194 activity, we performed infections after incubating activated cells for 10 days in IL-2 (Kinoshita et al., 1998). Under these conditions we obtained a 50% inhibition of the pre-drug isolate using 10 μ M concentration of CNI-H1194, while AZT (1 μ M) almost completely inhibited virus replication (Fig. 4D). However, CNI-H1194 was as effective in inhibiting pre-virus as post-virus, while AZT was not. Indeed, replication of AZT-resistant isolate was inhibited by AZT by only 30%, while CNI-H1194 demonstrated the same inhibitory activity as observed with the parental, AZT-sensitive, virus (Fig. 4D). These results indicate that arylene bis(methylketone) compounds, despite their binding to RT (Popov et al., 1996), do not antagonize the effect of nucleoside inhibitors of RT. One possible explanation of this result is that CNI-H1194 and

RT inhibitors interact with different parts of the RT molecule. This interpretation is consistent with negligible activity of CNI-H1194 as a RT inhibitor and suggests that CNI-H1194 binds to RT outside of the active center.

CNI-H1194 potentiates the effect of protease inhibitor. In addition to RT inhibitors, inhibitors of HIV protease are important components of the drug cocktail used in HAART. We therefore tested anti-HIV activity of a combination of CNI-H1194 and nelfinavir, a protease inhibitor kindly provided by Agouron Pharmaceuticals, Inc. (La Jolla, CA). As shown in Fig. 5, the presence of CNI-H1194 at a 100-nM concentration (EC_{50}) substantially potentiated the anti-HIV activity of nelfinavir. Again, cell viability in uninfected drug-treated cultures was \approx 98%. Although virus replication varied significantly in cultures prepared from peripheral blood of different donors, the inhibitory effect was rather consistent.

Results presented above suggest that nuclear import inhibitors of the arylene bis(methylketone)

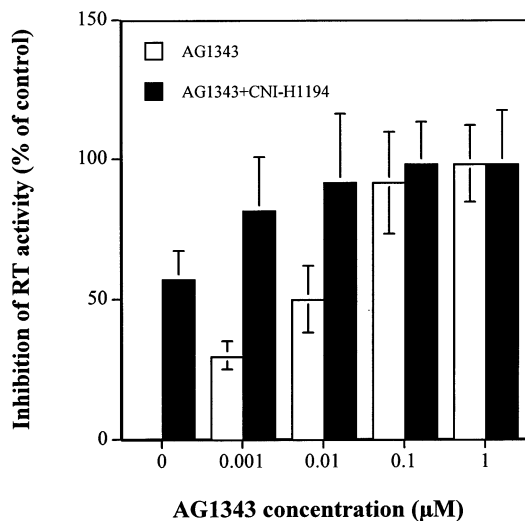


Fig. 5. Anti-HIV activity of CNI-H1194 combination with protease inhibitor. Experiment was performed with macrophage cultures infected with HIV-1_{ADA} as described in Fig. 4, except that protease inhibitor nelfinavir (AG1343) was used instead of RT inhibitors.

class of compounds might further improve the potency of HAART. The step of nuclear import follows reverse transcription and precedes integration, and the product of each reaction is the immediate substrate for the subsequent one. Therefore, one can expect a cooperative effect of RT, IN, and nuclear import inhibitors. Addition of the latter two classes of compounds to currently used anti-HIV drug cocktails may help achieve a complete long-term suppression of viral replication.

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