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Nitric Oxide Inhibits HIV-1 Replication in Human Astrocytoma Cells

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Astroglial cells represent a target for HIV infection in the central nervous system. In astrocytes, HIV infection is poorly productive, being characterized by a persistent state of viral latency. However, activation of the nuclear factor NF-κB and its binding to HIV long terminal repeat (LTR) can induce HIV replication. Moreover, nitric oxide (NO) can affect NF-κB activation in glial cells. Therefore, we hypothesize that NO may reduce HIV replication in human astroglial cells by inhibiting HIV-1 LTR transcriptional activity. In this respect, we show that NO donors reduce viral replication in HIV-1-infected human astrocytoma T67 cells, taken as an astroglial model. Furthermore, using transfected T67 cells, we demonstrate that NO donors inhibit HIV-1 LTR transcriptional activity. These results suggest that the use of NO-releasing drugs may represent a potential, novel approach in inhibiting HIV replication in the central nervous system.

Key Words: **nitric oxide; HIV-1 replication; AIDS dementia complex; glial cells.**

AIDS dementia complex (ADC) is associated with HIV replication in the central nervous system occurring following the infection of cells, such as infiltrating macrophages, resident brain microglia, and astrocytes [1, 2]. In astroglial cells, HIV infection is poorly productive, being typified by a persistent state of infection in which few or no viral structural antigens are expressed [3]. However, HIV replication may be induced by the activation and binding of the nuclear factor NF-κB to the consensus sequence on HIV long terminal repeat (LTR) [4, 5].

Recently, it has been reported that nitric oxide (NO) can affect NF-κB activation [6] and DNA binding through Cys62 S-nitrosylation [7]. Moreover, endogenous NO is able to inhibit the HIV-1 LTR transcription in the human lymphoblastoid T cell line [8]. Based on these considerations, we hypothesize that NO may reduce HIV replication in human astroglial cells by inhibiting HIV-1 LTR transcriptional activity. In this respect, using HIV-1-infected human astrocytoma T67 cells, taken as an astroglial model, we tested the action of drugs releasing NO on viral replication. Furthermore, using transfected T67 cells, we analyzed the effect of NO-releasing drugs on the HIV-1 LTR transcriptional activity. Here, we demonstrate that NO reduces HIV-1 replication and inhibits HIV-1 LTR transcription in human astrocytoma cells. Therefore, the present results may represent a potential, useful tool in keeping HIV replication in human central nervous system suppressed.

MATERIALS AND METHODS

Human astrocytoma T67 cells were obtained from explant of III WHO gemistocytic astrocytoma and characterized in our laboratory [9]. T67 cells were 6-well plated (5×10^5 cells/well) and adsorbed overnight at 37°C with HIV-1 BaL (100 $TCID_{50}$/ml), isolated from an infected patient. After adsorption, the unbound viruses were removed by three gentle washes with serum-free medium, and fresh medium was added to each plate for further incubation at 37°C.

HIV-1 replication was determined in duplicate samples by measuring p24 protein in cell supernatants with a p24 ELISA kit (NEN, Life Science Products, Boston, MA). Cell survival at day 9 after infection was 90%, as determined by counting the number of the cells not including trypan blue. Results are expressed as pg/ml after normalization to the cell number. Each p24 value represents the mean ± SEM of three independent measurements.

In ptzIIICAT plasmid, the bacterial gene chloramphenicol acetyltransferase (CAT) is directed by the HIV-1 LTR [10, 11]. T67 cells (1×10^5) were transiently transfected with ptzIIICAT (2.0 μg), using Superfect transfection reagent as specified by the manufacturer (Qiagen GmbH, Hilden, Germany). At 3-h transfection, cells were

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Abbreviations used: AIDS, acquired immunodeficiency syndrome; ADC, AIDS dementia complex; HIV-1, human immunodeficiency virus-1; NO, nitric oxide; NOR-3, (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide; SIN-1, 3-morpholinosydnonimine; NOC-18, 3,3-bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase.

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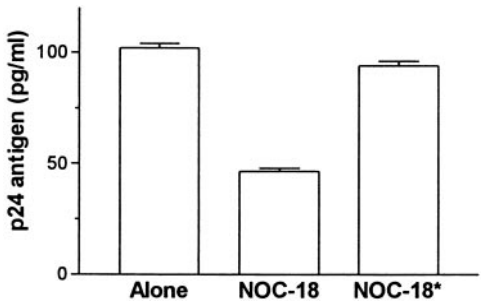

FIG. 1. Nitric oxide decreased p24 antigen release by human T67 astrocytoma cells. Levels of p24 were determined in the supernatants of HIV-1-infected T67 astrocytoma cells (5×10^5 cells). When HIV-1-infected T67 cells were incubated with NOC-18 (1.0 mM), a decrease in p24 antigen release at day 9 after HIV infection was observed. NO-deprived NOC-18 (NOC-18*, 1.0 mM) did not affect p24 antigen levels. Results are expressed as picograms (pg) of p24 per milliliter (ml) of supernatant after normalization to the cell number. Each bar represents the mean ± SEM of three experiments.

treated with different NO donors (NOC-18; SIN-1; NOR-3). At 48 h after transfection, the cells were harvested and lysed. The protein concentration in the cell extracts was determined by the method of Bradford [12]. Equivalent amounts of protein were assayed in duplicate samples for quantitative determination of CAT using a CAT ELISA kit (Boehringer-Mannheim Italia, Monza, Italy). Transfection efficiency was evaluated by transfection with pCMV-βgal.

NO donors (±)-(E)-4-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (NOR-3), 3-morpholinosydnonimine (SIN-1), and 3,3-bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene (NOC-18) were purchased from Sigma-Aldrich S.r.l. (Milan, Italy). HIV-1 BaL was kindly provided by Dr. G. Antonelli (Institute of Virology, University of Rome "La Sapienza," Italy). The ptzIIICAT plasmid was kindly provided by Dr. A. Corallini and Dr. A. Caputo (Department of Experimental and Diagnostic Medicine, University of Ferrara, Italy).

RESULTS AND DISCUSSION

To verify the effect of NO donors on HIV replication in glial cells, we used human T67 astrocytoma cells which are susceptible to HIV-1 infection, as previously described [1]. Under our experimental conditions, HIV-1 BaL-infected T67 cells released p24 antigen into cell supernatants, as detected 9 days after infection (Fig. 1). Preincubation of HIV-infected T67 cells with 1.0 mM NOC-18, an NO donor characterized by a slow NO release ($t_{1/2}$ = 3400 min), caused a significant reduction of p24 levels in the cell supernatants (Fig. 1). NO-deprived NOC-18 (1.0 mM), obtained after 1 week preincubation at 25°C for NO prerelease, did not affect p24 antigen production in HIV-infected T67 cell supernatants (Fig. 1), thus demonstrating that the effect of NOC-18 on viral replication was mediated by NO release.

Recently, it has been demonstrated that, by affecting NF-κB activation, NO inhibits the HIV-1 LTR transcriptional activation in the human lymphoblastoid T cell line [8]. In the central nervous system, for example, NO has been reported to hinder the productive infection of animal viruses, through the inhibition of viral RNA synthesis, protein accumulation, and virus release from infected cells [13, 14]. Also, in glial cells, exogenous NO has been demonstrated to affect the activation of NF-κB [6, 7], an important transcriptional factor involved in HIV replication [4, 5]. By using human T67 astrocytoma cells as a model of glial cells, we analyzed the effect of NO donors on the HIV-1 transcriptional activity. In this respect, T67 cells were transfected with chloramphenicol acetyltransferase (CAT) reporter gene controlled by the HIV-1 LTR promoter. When transfected-T67 cells were treated with NOR-3 (characterized by $t_{1/2}$ = 45 min for NO release, at pH 6.5 and 25.0°C) for 48 h, a dose-dependent inhibition of CAT expression was observed (Fig. 2). CAT expression was also inhibited by NOC-18 and SIN-1 (each used at 1.0 mM), as shown in Fig. 2. Furthermore, NO-deprived NOR-3 (1.0 mM), as obtained after pre-incubation at 25°C for 72 h for pre-release of NO, did not affect CAT expression (Fig. 2), thus confirming that the effect of NOR-3 was mediated by NO release.

The results we obtained in human glial cells with exogenous NO are consistent with data in the literature reporting that endogenous NO inhibited the HIV-1 LTR transcriptional activation in the human lymphoblastoid T cell line [8]. However, NO may also affect other pathways. In this respect, HIV-1 replication requires the cysteine-containing viral-encoded

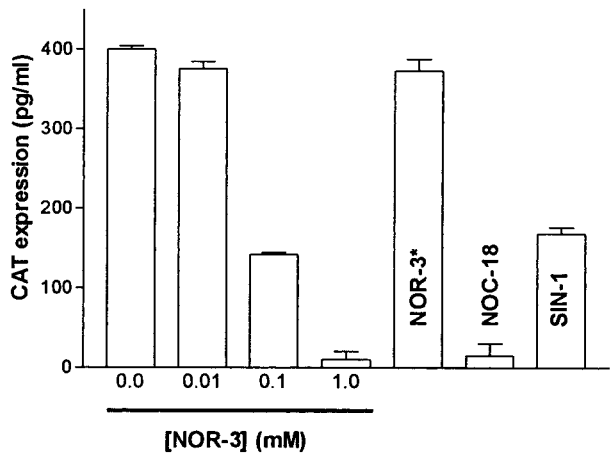

FIG. 2. HIV-1 LTR-driven CAT expression was inhibited by NO donors. CAT levels were measured in cell extracts of T67 cells transfected with 2.0 μg of ptzIIICAT. When ptzIIICAT-transfected cells were incubated with NO donor NOR-3 (from 0.1 to 1.0 mM), a dose-dependent inhibition in CAT expression at 48 h after transfection was observed. 1.0 mM NO-deprived NOR-3 (NOR-3*) did not affect CAT levels. Also, NO donors NOC-18 and SIN-1 (each used at 1.0 mM) significantly decreased CAT expression at 48 h after transfection. Results are expressed as picograms (pg) of CAT per milliliter (ml) of cell-extract and are compared to the basal expression in ptzIIICAT-transfected cells incubated in medium alone. Cell extracts were prepared, normalized for total protein, and assayed for CAT expression. Each bar represents the mean ± SEM of three experiments.

protease which cleavages precursor polyproteins, leading to maturation of infectious virions. Recently, we have reported that NO donors are able to inhibit HIV-1 protease activity through S-nitrosylation of cysteine residue(s) [15], thus providing a further possible mechanism for inhibition of HIV-1 replication.

The present results indicate that exogenous NO reduces HIV-1 replication in astrocytoma cell culture, thereby representing a possible, novel class of molecules for reducing HIV replication in the central nervous system. It is worth noting that molsidomine (SIN-10), a stable precursor metabolized in the liver to SIN-1, is clinically used in the treatment of cardiovascular disorders as a NO-releasing drug [16]. Therefore, the use of NO-releasing drugs appears to be a potential, useful tool in regulating HIV-1 replication *in vivo.*

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REFERENCES

1. Persichini, T., Mancino, G., Cappelli, G., Colizzi, V., and Lauro, G. M. (1997) *NeuroReport* **8,** 1897–1901.
2. Lipton, S. A., and Gendelman, H. E. (1995) *N. Engl. J. Med.* **332,** 934–940.
3. Tornatore, C., Meyers, K., Atwood, W., Conant, K., and Major, E. (1994) *J. Virol.* **68,** 93–102.
4. Griffin, G. E., Leung, K., Folks, T. M., Kunkel, S., and Nabel, G. J. (1989) *Nature* **339,** 70–73.
5. Atwood, W. J., Tornatore, C. S., Traub, R., Conant, K., Drew, P. D., and Major, E. O. (1994) *AIDS Res. Hum. Retroviruses* **10,** 1207–1211.
6. Colasanti, M., Persichini, T., Menegazzi, M., Mariotto, S., Giordano, E., Caldarera, C. M., Sogos, V., Lauro, G. M., and Suzuki, H. (1995) *J. Biol. Chem.* **270,** 26731–26733.
7. Matthews, J. R., Botting, C. H., Panico, M., Morris, H. R., and Hay, R. T. (1996) *Nucleic Acids Res.* **24,** 2236–2246.
8. Sekkai, D., Aillet, F., Israel, N., and Lepoivre, M. (1998) *J. Biol. Chem.* **273,** 3895–3900.
9. Lauro, G. M., Di Lorenzo, N., Grossi, M., Maleci, A., and Guidetti, B. (1986) *Acta Neuropathol. (Berlin)* **69,** 278–282.
10. Campioni, D., Corallini, A., Zauli, G., Possati, L., Altavilla, G., and Barbanti-Brodano, G. (1995) *AIDS Res. Hum. Retroviruses* **11,** 1039–1048.
11. Beauparlant, P., Kwon, H., Clarke, M., Lin, R., Sonenberg N., Wainberg, M., and Hiscott, J. (1996) *J. Virol.* **70,** 5777–5785.
12. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
13. Karupiah, G., Xie, Q-w., Buller, R. M. L., Nathan, C., Duarte, C., and MacMicking, J. D. (1993) *Science* **261,** 1445–1448.
14. Lin, Y-L., Huang, Y-L., Ma, S-H., Yeh, C-T., Chiou, S-Y., Chen, L-K., and Liao, C-L. (1997) *J. Virol.* **71,** 5227–5235.
15. Persichini, T., Colasanti, M., Lauro, G. M., and Ascenzi, P. (1998) *Biochem. Biophys. Res. Commun.* **250,** 575–576.
16. Martindale, J. (1996) *in* The Extra Pharmacopoeia (James, E., and Reynolds, F., Eds.), 31st ed., Pharmaceutical Press, London.