

Research article

Open Access

Peroxynitrite decomposition catalyst prevents apoptotic cell death in a human astrocytoma cell line incubated with supernatants of HIV-infected macrophages

Carolina Muscoli¹, Daniela Salvemini², Donatella Paolino³, Michelangelo Iannone⁴, Ernesto Palma¹, Antonio Cufari¹, Domenicantonio Rotiroti¹, Carlo Federico Perno^{5,6}, Stefano Aquaro⁵ and Vincenzo Mollace*¹

Address: ¹Faculty of Pharmacy, University of Catanzaro "Magna Graecia", Roccelletta di Borgia, Catanzaro, Italy, ²MetaPhore Pharmaceuticals, Inc., 1910 Innerbelt Business Center Drive, St Louis, MO 63114, USA, ³Department of Pharmaceutical Sciences, University of Catania, Italy, ⁴IBAF Centre, CNR, Roccelletta di Borgia, Catanzaro, Italy, ⁵Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Rome, Italy and ⁶IRCCS "L. Spallanzani", Rome, Italy

E-mail: Carolina Muscoli - muscoli@uniroma3.it; Daniela Salvemini - dsalvemini@metaphore.com; Donatella Paolino - fresta@unicz.it; Michelangelo Iannone - iannone@cs.cnr.it; Ernesto Palma - palma@unicz.it; Antonio Cufari - acufari@libero.it; Domenicantonio Rotiroti - rotiroti@unicz.it; Carlo Perno - cf.perno@uniroma2.it; Stefano Aquaro - cf.perno@uniroma2.it; Vincenzo Mollace* - mollace@libero.it

*Corresponding author

Published: 16 September 2002

Received: 21 May 2002

BMC Neuroscience 2002, 3:13

Accepted: 16 September 2002

This article is available from: <http://www.biomedcentral.com/1471-2202/3/13>

© 2002 Muscoli et al; licensee BioMed Central Ltd. This article is published in Open Access: verbatim copying and redistribution of this article are permitted in all media for any non-commercial purpose, provided this notice is preserved along with the article's original URL.

Keywords: Peroxynitrite, astroglial cells, therapy, AIDS dementia complex, FeTPMS

Abstract

Background: Oxidative stress has shown to contribute in the mechanisms underlying apoptotic cell death occurring in AIDS-dementia complex. Here we investigated the role of peroxynitrite in apoptosis occurring in astroglial cells incubated with supernatants of HIV-infected human primary macrophages (M/M).

Results: Flow cytometric analysis (FACS) of human cultured astrocytes shortly incubated with HIV-1-infected M/M supernatants showed apoptotic cell death, an effect accompanied by pronounced staining for nitrotyrosine (footprint of peroxynitrite) and by abnormal formation of malondialdehyde (MDA). Pretreatment of astrocytes with the peroxynitrite decomposition catalyst FeTPMS antagonized HIV-related astrocytic apoptosis, MDA formation and nitrotyrosine staining.

Conclusions: Taken together, our results suggest that inhibition of peroxynitrite leads to protection against peroxidative stress accompanying HIV-related apoptosis of astrocytes. Overall results support the role of peroxynitrite in HIV-related programmed death of astrocytes and suggest the use of peroxynitrite decomposition catalyst to counteract HIV-1-related neurological disorders.

Background

Evidence exists suggesting that HIV-1-infected patients are under chronic oxidative stress [1,2]. Major causes of the increased concentration of free radicals are: i) a depletion of protective systems (glutathione peroxidase, superoxide dismutase, vitamin E, selenium), and an increased production of free radicals (superoxide anion, hydroxyl radical) associated with the activation of lymphocytes and M/M; ii) chronic inflammation; iii) increased polyunsaturated fatty acid concentration and lipid peroxidation; iv) the direct or indirect effects of several opportunistic pathogens [1,3]. In addition, elevated serum levels of hydroperoxides and malondialdehyde, which are both indicative of oxidative stress, have also been found in asymptomatic HIV-1-infected patients early in the course of the disease [2]. Despite the demonstrated role of free radicals in AIDS-dementia complex, the mechanism underlying HIV-related oxidative damage of CNS cells is still unknown. Recently, it has been shown that astroglial apoptosis play a key role in the neurological progression of HIV-1 associated dementia [4] and furthermore, incubation of astroglial cells with supernatants of HIV-infected macrophages leads to astrocytic oxidative stress and then to apoptotic cell death. This effect is attenuated by novel non peptidic superoxide dismutase mimetics, thus supporting that an abnormal generation of superoxide anions may contribute in HIV-related apoptosis [2,3]. Evidence exists that HIV infection is accompanied by simultaneous activation of free radical species in CNS cells other than superoxide anions, such as nitric oxide (NO). Since NO is able to combine with superoxide anions to generate peroxynitrite, a nitrogen free radical [5] accounted to produce relevant peroxidative HIV-related damage in CNS cells, it is likely that abnormal release of peroxynitrite may play a role in the apoptotic cell death which occurs when incubating astroglial cells with supernatants of HIV-infected M/M [6]. The present experiments have been performed in order to evaluate the role of peroxynitrite in the apoptotic cell death of astroglial cells incubated with supernatants of HIV-1-infected M/M and ascertain the protective effect of FeTMPS, a novel peroxynitrite decomposition catalyst on HIV-1-related apoptosis of astroglial cells.

Results

Effect of HIV-1 infected macrophage supernatant on astroglial cells

Time-course studies revealed that astroglial cell apoptosis occurred between 6 and 8 days after addition of the supernatant from HIV-1 infected macrophages. Thus results will be shown at day 8. In particular, when astrocytes were incubated with supernatants of HIV-infected M/M, a dramatic reduction of cell viability was seen at day 8 by flow cytometric analysis (FACS). In sharp contrast, supernatants of mock-infected M/M only marginally affected astrocytes viability. The cytopathic effect observed in

astrocytes exposed to HIV-1-infected M/M supernatants was mainly related to apoptosis. Indeed, FACS analysis showed apoptosis in 49% and 7% of astrocytes exposed to HIV-1-infected M/M or mock-infected cells, respectively (Fig. 1). Indeed, 121 nmol of MDA/mg protein of cell homogenate were found in astroglial cells at day 8 after exposure to HIV-1-infected M/M supernatants, with an increment of MDA production of about 9-fold compared to controls (Fig. 2). Neither apoptotic phenomena, nor MDA over production were generated after incubation of astroglial cells with supernatants from mock-infected M/M (Fig. 1 and 2).

When cells were treated with supernatants of HIV-1-infected M/M, a corresponding increase in the number of cells that stained with nitrotyrosine-specific mouse antiserum was observed at day 2 (Fig. 3), indicating that an HIV-related peroxynitrite formation occurred in astroglial cells. No apoptosis was seen at day 2.

Effect of FeTMPS on astroglial cells following incubation with supernatants from HIV-1 infected macrophages

Dose responses with FeTMPS were performed (1–30 μ M) and maximal effects were obtained between 10 and 30 μ M. Results at the highest dose used are shown. Pretreatment of astrocytes with peroxynitrite decomposition catalyst FeTMPS strongly antagonized apoptosis induced by HIV-1-infected M/M supernatants. FeTMPS showed a potent dose-dependent effect as assessed by FACS analysis at day 8, only 10% of astroglial cells exposed to supernatants from HIV-1-infected M/M, and treated with 30 μ M FeTMPS, showed signs of apoptosis, compared to 49% of untreated astrocytes similarly exposed to HIV-1-infected M/M supernatants (Fig. 1). In addition, MDA overproduction was significantly decreased by FeTMPS: 121 and 38 nmol MDA/mg protein of cell homogenate were measured in not-treated and FeTMPS-treated astroglial cell cultures respectively, at day 8 after the exposure to HIV-1-infected M/M supernatants (Fig. 2). In sharp contrast, treatment of astrocytes with H₂TMPS (an inactive analogue compound of FeTMPS; 30 μ M), or FeCl₃ (30 μ M) failed to prevent both apoptosis and MDA formation (Fig. 1 and 2). On the other hand, nitrotyrosine staining was decreased using the active catalyst FeTMPS (Fig. 3). FeTMPS did not affect, when incubated in the absence of M/M supernatants, viability of astroglial cells at concentration up to 50 μ M (data not shown).

Discussion

The alteration of the homeostasis induced by HIV-1 infection, with consequent production of toxic factors, is claimed to be the main cause of neuronal damage during AIDS. In particular, the release of some coating component of HIV-1, such as gp120 glycoprotein or Tat, by HIV-1-infected M/M produces both direct and indirect effects

Figure 1

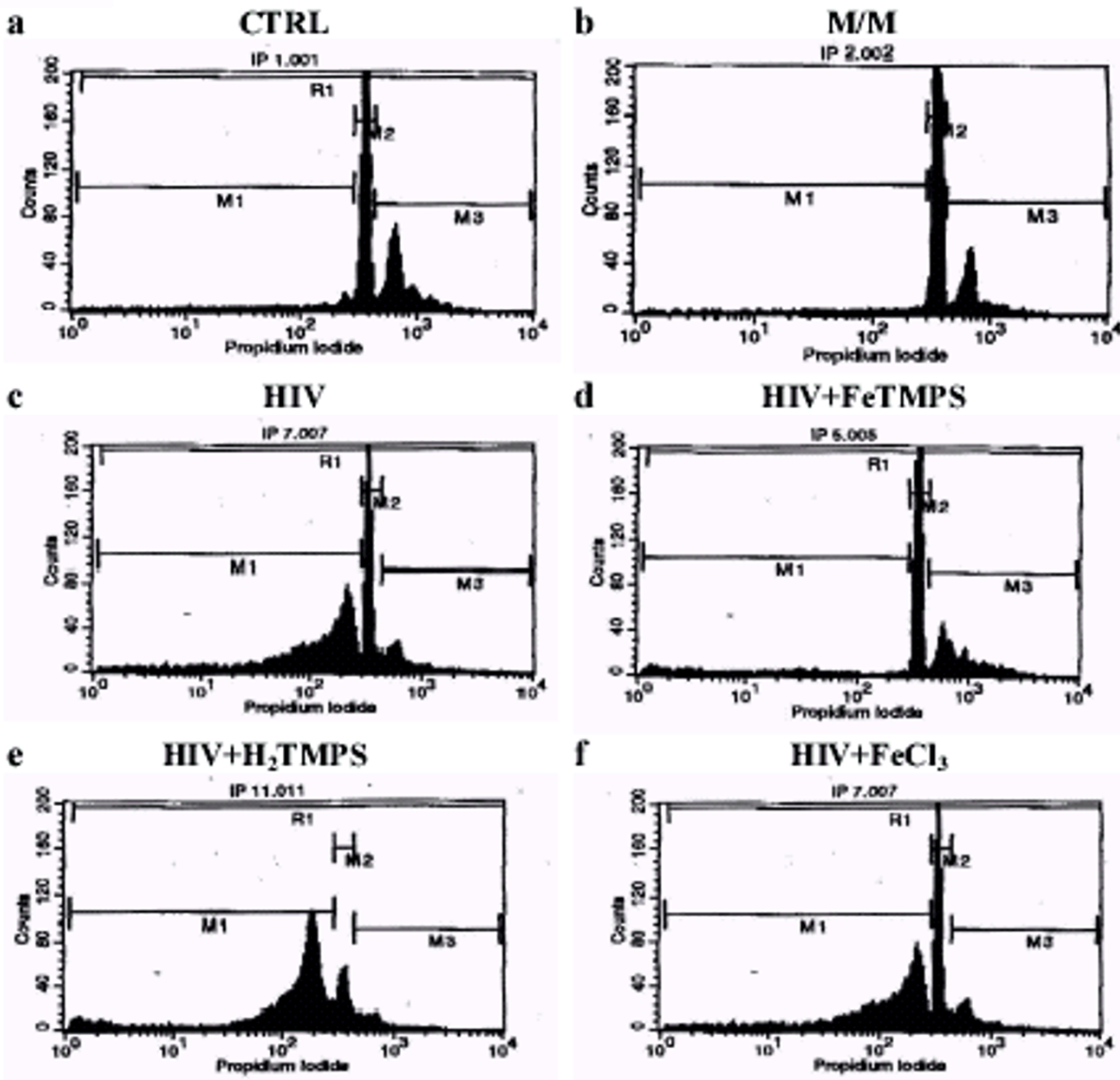


Figure 1

FeTMPS prevents apoptosis in astroglial cells induced by HIV-1-infected M/M. Supernatants from HIV-1 infected M/M (M/M + HIV) produces apoptotic cell death of astroglial cells (c) greater than supernatants from mock-infected (M/M) (b) as evaluated by FACS analysis 8 days after exposure to supernatants. FeTMPS (d), but not H₂TMPS (e) or FeCl₃ (f), antagonized this effect.

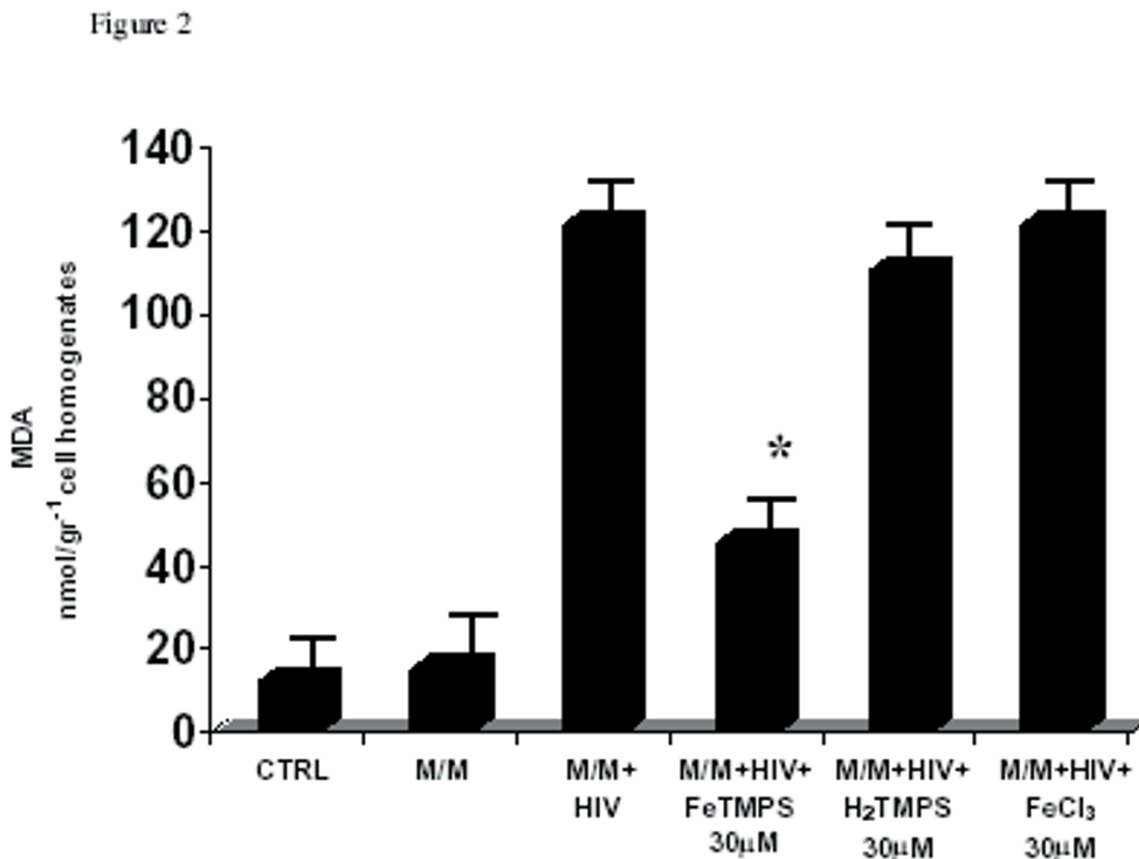


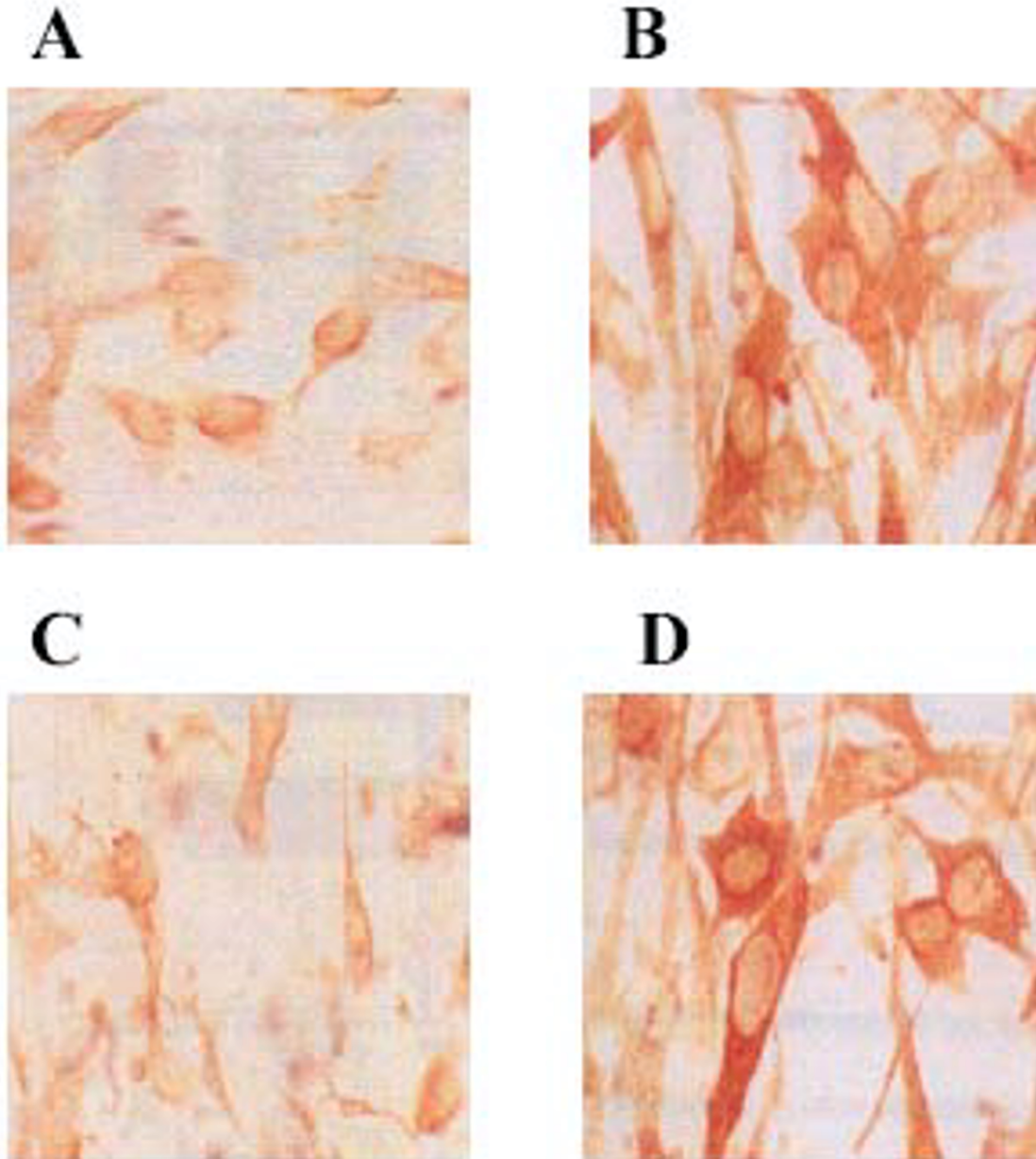
Figure 2 Malondialdehyde increases within astroglial cells incubated with supernatants of HIV-infected M/M (M/M + HIV) but not after exposure to supernatants from mock-infected M/M (M/M). FeTMPS antagonized MDA overproduction, while H₂TMPS or FeCl₃ failed to antagonize lipid peroxydation. Values are the mean out of four independent experiments. Error bars represent standard deviations. * P < 0.05 FeTMPS-treated vs FeTMPS-untreated astroglial cells

in the central nervous system (CNS) [7–9]. Indeed, gp120, Tat and TNF α are capable to produce apoptotic cell death via cytokine release or caspase activation [10–13]. In vitro incubation of glial cells and neurons with gp120 or Tat leads to release of large amount of free radicals and, in turn, to neurodegenerative effects by activation of both cytokine network and excitatory amino acid NMDA receptor sensitization [14–17].

Previous studies have shown that HIV-1-infected M/M are able to induce apoptosis of T-lymphocytes or astroglial cells via overproduction of several factors, including pros-

taglandins, CD95-ligand, and free-radicals [18,19]. Furthermore, arachidonic acid produced by infected M/M has been reported able to cause neuronal damage [14]. Finally, it has been shown that HIV-1 infection induces a heavy perturbation of oxidative status of M/M, including increased production of MDA, and decreased synthesis of endogenous glutathione [20] thus indicating that the interaction of HIV-1 with macrophages/microglial cells and the release of HIV-1 components into CNS represents apoptotic cell death of brain cells in neuroAIDS, via ROS overproduction. This is also shown by our experiments where we found that the HIV-supernatants-mediated ap-

Figure 3

**Figure 3**

Nitrotyrosine staining in astroglial cells either untreated (a) or pre-treated with supernatants of HIV-1-infected macrophages (b,c,d). In particular, the supernatant of HIV-infected macrophages enhanced the immunocytochemical expression of nitrotyrosine (b), indicating an increased production of peroxynitrite. Incubation of cells with FeTMPS (c) but not with the inactive catalyst H₂TMPS (d) inhibited HIV-related peroxynitrite formation in astrocytes. These are representative photomicrographs (optical microscopy 40×) out of four independent experiments.

optosis of astroglial cells was accompanied by an increased generation of free radicals, in particular of peroxynitrite, the reaction product of nitric oxide and superoxide [5]. The role of peroxynitrite in our study was assessed by the use of the peroxynitrite decomposition catalyst, FeTMPS which catalytically decompose peroxynitrite to nitrate [21,22]. In previous studies, FeTMPS but not H₂TMPS (inactive analog) [21] was found to be protective against peroxynitrite-mediated damage *in vitro* as well as in several animals models of acute and chronic inflammation [23–26]. Importantly, FeTMPS does not interact with NO making it an important tool to assess peroxynitrite-mediated damage [23–25].

Our results suggest that overproduction of peroxynitrite, possibly via the release of pro-inflammatory substances by HIV infected macrophages/microglial cells, may contribute in the pathophysiological mechanisms underlying astroglial apoptotic cell death in neuro-AIDS. In addition, due to their innovative pharmacological profile, the use of novel non peptidyl peroxynitrite decomposition catalyst, such as FeTMPS, may represent the basis for alternative and efficient strategies in the treatment of neuroAIDS.

Methods

Cell cultures

Human primary macrophages

Peripheral blood mononuclear cells (PBMCs) were obtained from the blood of healthy seronegative donors by separation over Ficoll-Hypaque gradient. After separation, PBMCs were seeded at a density of 6×10^6 cells/ml in 25 cm² plastic flasks in RPMI 1640 with the addition of 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mM L-glutamine, and 20% heat-inactivated, mycoplasma- and endotoxin-free fetal calf serum (FCS) (hereinafter called complete medium). Cells were incubated at 37°C in humidified air containing 5% CO₂. After 5 days of culture, non-adherent cells were removed by repeated washing with warm medium. Macrophages obtained with this method resulted in >95% of purity analyzed by flow cytometry analysis.

Human astrocytoma cell line

The astroglial cell line Lipari was derived from a 51-year old male patient who presented a large right front-temporal mass (astrocytoma) [27], these cells are not permissive to HIV-1 infection [28]. Cells were expanded and cultured by seeding them in 25 cm² plastic flasks at a density of 0.7×10^6 cells/flask in complete medium, and incubated at 37°C in humidified air containing 5% CO₂.

HIV-1 strain and M/M infection

A monocytotropic strain of HIV-1, named HIV-1Ba-L, was used in all experiments. Characteristics and genomic sequence of this strain have been previously described [29,30]. The virus was expanded in M/M, collected, fil-

tered and stored at -80°C before use. Its concentration was 2.1×10^8 genomes, corresponding to 35 ng of p24 gag antigen (Ag), and 5,000 tissue culture infectious doses 50% per ml (TCID₅₀/ml) as assessed by virus titration in M/M. Macrophages were challenged for 2 hours with 300 TCID₅₀/ml of virus, then extensively washed with warm medium to remove the excess of virus, and finally cultured in complete medium at the same conditions as before. Macrophages were washed and fed every 7 days with fresh complete medium. Supernatants of HIV-1-infected and mock-infected M/M were collected at day 14 after virus challenge, spun to remove cells and cellular debris, and stored at -80°C until use. Virus production was determined by the antigen-capture assay using a commercially available p24 gag Ag kit (Abbott Pomezia, Italy).

Challenge of astrocytes

Astrocytes were incubated with supernatants from HIV-1-infected M/M or mock-infected M/M for 4 hours at 37°C in humidified incubator. FeTMPS (30 µM), H₂TMPS (30 µM) or FeCl₃ (30 µM) were added immediately before exposure to supernatants of HIV-1-infected or mock-infected M/M. Four hours later, cells were carefully and repeatedly washed (at least 5 times) to remove M/M supernatants, and cultured in complete medium with and without a daily treatment with all the compounds used in this study for the duration of the experiment (6–8 days).

Flow cytometric analysis (FACS)

Astrocytes treated or not treated with supernatants of HIV-1-infected or mock-infected M/M were gently detached from plastic 6–8 days after virus challenge. Aliquots of 5×10^5 cells were centrifuged at $300 \times g$ for 5 min; pellets were washed with PBS, placed on ice, and overlaid with 0.5 ml of a hypotonic fluorochrome solution containing 50 µg/ml propidium iodide, 0.1% sodium citrate, and 0.1% Triton X-100. After gentle resuspension in this solution, astrocytes were left at 4°C for 30 min, in the absence of light, before analysis. Propidium iodide-stained cells were analyzed with a FACScan Flow Cytometer (Becton Dickinson), fluorescence was measured between 565 and 605 nm. The data were acquired and analysed by the Lysis II program.

Malondialdehyde (MDA) determinations

MDA has been used as a biochemical marker for lipid peroxidation and was measured by a method previously described [31]. In particular, levels of MDA were measured in astroglial cell homogenates 8 days after cell exposure to supernatants of HIV-1-infected M/M as indicated above. In particular, astroglial cells were frozen in liquid nitrogen, and homogenized in potassium chloride (1.15%). Chloroform (2 ml) was then added to each homogenate and then spun for 30 min. The organic layer of the sample was removed and dried under nitrogen gas and reconstituted

with 100 µl of saline. MDA generation was evaluated by the assay of thiobarbituric acid (TBA)-reacting compounds. In particular, the addition of a solution of 20 µl of sodium dodecyl sulphate (SDS; 8.1%), 150 µl of 20% acetic acid solution (pH3.5), 150 µl of 0.8% TBA and 400 µl of distilled water, produced a chromogenic product which was extracted in n-butanol and pyridine. The organic layer was removed and MDA was read at 532 nm and expressed as nmol MDA/mg protein of cell homogenate.

Immunocytochemical Staining

Immunocytochemical staining for nitrotyrosine was performed on treated or not treated astrocytes. Astrocytes were fixed with 4% paraformaldehyde dissolved in 0.1% phosphate buffer (pH 7.4). Nonspecific staining was blocked with 3% normal goat serum in 0.5 M Tris-HCl, pH 7.4 containing 0.2% Tween 20 for 1 h at room temperature. All subsequent incubations were carried out in this buffer. For detection of nitrotyrosine immunoreactivity, cells were incubated for 16 h at 4°C with an anti-nitrotyrosine monoclonal Ab (Cayman, 1:500) at 4°C. The primary Ab was then removed, and the cells were exposed to the secondary Ab, which was a biotinylated goat anti-mouse Ig (IgG), and avidin-biotin-horseradish peroxidase (VECTASTAIN Elite ABC Kit; Vector Laboratories), followed by diaminobenzidine.

Statistical analysis

Statistical significance and standard deviations were assessed using the Student t test.

Authors' contributions

Author 1 initials carried out the cytometric analysis, the immunocytochemistry, participated in the sequence alignment and drafted the manuscript. Author 2 participated in the design of the study and drafted the manuscript. Authors 3 initials carried out the biochemical analysis. Authors 4 and 5 initials participated in the sequence alignment. Author 6 performed the statistical analysis. Authors 7 and 8 conceived of the study. Author 9 initials carried out the virology study. Author 10 participated in the design of the study and in its coordination.

Acknowledgements

This work was supported by Ministry of University and Scientific Research (MURST; COFIN 2000 and COFIN 2001), Italian National Council for Research (CNR), by the European Found for Regional Development (POP 94/99), by grants from the AIDS Project (ISS), Italy, by the Ricerca Corrente of the Italian Ministry of Health, and by Biomed Project of European Community. Our thanks go to Mrs Tania Guenci, and Mr. Giovanni Politi (University of Rome "Tor Vergata", Rome, Italy) for their excellent technical support.

References

1. Chiueh CC, Rauhala P: **The redox pathway of S-nitrosoglutathione, glutathione and nitric oxide in cell to neuron communications.** *Free Radic Res* 1999, **31**:641-650

2. Mollace V, Nottet HSLM, Clayette P, Turco C, Muscoli C, Salvemini D, Perno CF: **Oxidative stress and neuroAIDS: triggers, modulators and novel antioxidants.** *Trends Neurosci* 2001, **24**:411-416
3. Mollace V, Salvemini D, Riley DP, Muscoli C, Granato T, Masuelli L, Modesti A, Rotiroti D, Nistico R, Bertoli A, Perno CF, Aquaro S: **The contribution of oxidative stress in apoptosis of human-cultured astroglial cells induced by supernatants of HIV-1-infected macrophages.** *J Leukoc Biol* 2002, **71**(1):65-72
4. Thompson KA, McArthur JC, Wesselingh SL: **Correlation between neurological progression and astrocyte apoptosis in HIV-associated dementia.** *Ann Neurol* 2001, **49**(6):745-752
5. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA: **Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide.** *Proc Natl Acad Sci USA* 1990, **87**:1620-1624
6. Boven LA, Gomes L, Hery C, Gray F, Verhoef J, Portegies P, Tardieu M, Nottet HS: **Increased peroxynitrite activity in AIDS dementia complex: implications for the neuropathogenesis of HIV-1 infection.** *J Immunol* 1999, **162**:4319-4327
7. Nottet HS, Gendelman HE: **Unraveling the neuroimmune mechanisms for the HIV-1-associated cognitive/motor complex.** *Immunol Today* 1995, **16**:441-4489
8. Price RW: **The cellular basis of central nervous system HIV-1 infection and the AIDS dementia complex.** *J Neuro-AIDS* 1996, **1**:1-20
9. Shi B, Raina J, Lorenzo A, Busciglio J, Gabuzda D: **Neuronal apoptosis induced by HIV-1 Tat protein and TNF-alpha: potentiation of neurotoxicity mediated by oxidative stress and implications for HIV-1 dementia.** *J Neurovirol* 1998, **4**(3):281-290
10. Bagetta G, Corasaniti MT, Paoletti AM, Berliocchi L, Nisticò R, Giammarioli AM, Finazzi-Agrò A: **HIV-1 gp120-induced apoptosis in the rat neocortex involves enhanced expression of cyclo-oxygenase type 2 (COX-2).** *Biochem Biophys Res Commun* 1998, **244**:819-824
11. Bagetta G, Corasaniti MT, Berliocchi L, Nisticò R, Giammarioli AM, Malorni W, Aloe L, Finazzi Agrò A: **Involvement of interleukin-1β in the mechanism of human HIV-1 recombinant protein gp120-induced apoptosis in the neocortex of rat.** *Neuroscience* 1999, **89**:1051-1066
12. Bagetta G, Corasaniti MT, Costa N, Berliocchi L, Finazzi-Agrò A, Nisticò G: **The human immunodeficiency virus type 1 glycoprotein gp120 reduces the expression of neuronal nitric oxide synthase in the hippocampus but not in the cerebral cortex and medial septal nucleus of rat.** *Neurosci Lett* 1997, **224**:75-78
13. Kruman II, Nath A, Mattson MP: **HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload, and oxidative stress.** *Exp Neurol* 1998, **154**(2):276-288
14. Mollace V, Nisticò G: **Release of nitric oxide from astroglial cells: a key mechanism in neuroimmune disorders.** *Adv Neuroimmunol* 1995, **5**:421-30
15. Dawson VL, Dawson TM, Uhl GR, Snyder SH: **Human immunodeficiency virus type 1 coat protein neurotoxicity mediated by nitric oxide in primary cortical cultures.** *Proc Natl Acad Sci USA* 1993, **90**:3256-3259
16. Mollace V, Colasanti M, Muscoli C, Lauro GM, Rotirorti D, Nisticò G: **The effect of nitric oxide on cytokine-induced release of PGE2 by human cultured astroglial cells.** *Br J Pharmacol* 1998, **124**:742-746
17. Bruce-Keller AJ, Barger SW, Moss NI, Pham JT, Keller JN, Nath A: **Pro-inflammatory and pro-oxidant properties of the HIV protein Tat in a microglial cell line: attenuation by 17 beta-estradiol.** *J Neurochem* 2001, **78**(6):1315-1324
18. Aquaro S, Panti S, Caroleo MC, Balestra E, Cenci A, Forbici F, Ippolito G, Mastino A, Testi R, Mollace V, Calio R, Perno CF: **Primary macrophages infected by human immunodeficiency virus trigger CD95-mediated apoptosis of uninfected astrocytes.** *J Leukoc Biol* 2000, **68**:429-43
19. Mastino A, Grelli S, Piacentini M, Oliverio S, Favalli C, Perno CF, Garaci E: **Correlation between induction of lymphocyte apoptosis and prostaglandin E2 production by macrophages infected with HIV.** *Cell Immunol* 1993, **152**:120-130
20. Palamara AT, Perno CF, Aquaro S, Bue MC, Dini L, Garaci E: **Glutathione inhibits HIV replication by acting at late stages of the virus life cycle.** *AIDS Res Hum Retroviruses* 1996, **12**:1537-1541

21. Stern MK, Jensen MP, Kramer K: **Peroxynitrite decomposition catalysts.** *J Am Chem Soc* 1996, **118**:8735
22. Shimanovich R, Groves JT: **Mechanisms of peroxynitrite decomposition catalyzed by FeTMPS, a bioactive sulfonated iron porphyrin.** *Arch Biochem Biophys* 2001, **387**:307-317
23. Misko TP, Highkin MK, Veenhuizen AW, Manning PT, Stern MK, Currie MG, Salvemini D: **Characterization of the cytoprotective action of peroxynitrite decomposition catalysts.** *J Biol Chem* 1998, **273**:15646-15653
24. Salvemini D, Wang ZQ, Stern MK, Currie MG, Misko TP: **Peroxynitrite decomposition catalyst: therapeutics for peroxynitrite-mediated pathology.** *Proc Natl Acad Sci* 1998, **95**:2659-2663
25. Salvemini D, Riley DP, Lennon PJ, Wang ZQ, Currie MG, Macarthur H, Misko TP: **Protective effects of a superoxide dismutase mimetic and peroxynitrite decomposition catalysts in endotoxin-induced intestinal damage.** *Br J Pharmacol* 1999, **127**:685-692
26. Ferdinandy P, Danial H, Ambrus I, Rothery RA, Schulz R: **Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure.** *Circ Res* 2000, **87**:241-247
27. Zupi G, Candiloro A, Laudonio N, Carapella C, Benassi M, Riccio A, Bellocchi M, Greco C: **Establishment, characterization and chemosensitivity of two human glioma derived cell lines.** *J Neurooncol* 1988, **6**:169-177
28. Aquaro S, Panti S, Caroleo MC, Balestra E, Cenci A, Forbici F, Ippolito G, Mastino A, Testi R, Mollace V, Calio R, Perno CF: **Primary macrophages infected by human immunodeficiency virus trigger CD95-mediated apoptosis of uninfected astrocytes.** *J Leukoc Biol* 2000, **68**:429-435
29. Cenci A, Perno CF, Menzo S, Clementi M, Erba F, Tavazzi B, Di Pierro D, Aquaro S, Calio R: **Selected nucleotide sequence of the pol gene of the monocyctotropic strain HIV type I BaL.** *AIDS Res Hum Retroviruses* 1997, **13**:629-632
30. Perno CF, Newcomb FM, Davis DA, Aquaro S, Humphrey RW, Calio R, Yarchoan R: **Relative potency of protease inhibitors in monocytes/macrophages acutely and chronically infected with human immunodeficiency virus.** *J Infect Dis* 1998, **178**:413-422
31. Ohkawa H, Ohishi H, Yagi K: **Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction.** *Anal Biochem* 1979, **95**:351-358

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMedcentral will be the most significant development for disseminating the results of biomedical research in our lifetime."

Paul Nurse, Director-General, Imperial Cancer Research Fund

Publish with **BMC** and your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours - you keep the copyright

Submit your manuscript here:

<http://www.biomedcentral.com/manuscript/>



BioMedcentral.com

editorial@biomedcentral.com