Neurobiology of Disease 34 (2009) 462–476

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

HIV-1 gp120 neurotoxicity proximally and at a distance from the point of exposure: Protection by rSV40 delivery of antioxidant enzymes

Jean-Pierre Louboutin [a,*], Lokesh Agrawal [a], Beverly A.S. Reyes [b], Elisabeth J. Van Bockstaele [b], David S. Strayer [a]

[a] Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA
[b] Department of Neurosurgery, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA 19107, USA

ARTICLE INFO

Article history:
Received 26 October 2008
Revised 26 February 2009
Accepted 4 March 2009
Available online 25 March 2009

Keywords:
Cu/Zn superoxide dismutase
Glutathione peroxidase
NeuroAIDS
Substantia nigra
Tyrosine hydroxylase

ABSTRACT

Toxicity of HIV-1 envelope glycoprotein (gp120) for substantia nigra (SN) neurons may contribute to the Parkinsonian manifestations often seen in HIV-1-associated dementia (HAD). We studied the neurotoxicity of gp120 for dopaminergic neurons and potential neuroprotection by antioxidant gene delivery. Rats were injected stereotaxically into their caudate-putamen (CP); CP and (substantia nigra) SN neuron loss was quantified. The area of neuron loss extended several millimeters from the injection site, approximately 35% of the CP area. SN neurons, outside of this area of direct neurotoxicity, were also severely affected. Dopaminergic SN neurons (expressing tyrosine hydroxylase, TH, in the SN and dopamine transporter, DAT, in the CP) were mostly affected: intra-CP gp120 caused approximately 50% DAT+ SN neuron loss. Prior intra-CP gene delivery of Cu/Zn superoxide dismutase (SOD1) or glutathione peroxidase (GPx1) protected SN neurons from intra-CP gp120. Thus, SN dopaminergic neurons are highly sensitive to HIV-1 gp120-induced neurotoxicity, and antioxidant gene delivery, even at a distance, is protective.

Introduction

Human Immunodeficiency Virus (HIV)-associated Dementia (HAD), the most severe form of HIV-related CNS dysfunction, and the most common cause of dementia in adults under 40 was estimated to affect as many as 30% of patients with advanced Acquired Immune Deficiency Syndrome (AIDS) (Mattson et al., 2005; Major et al., 2000). It has become less common since Highly Active AntiRetroviral Therapy (HAART) was introduced (McArthur et al., 1993). This reduction probably reflects better ongoing entry of HIV-infected cells from the blood into the central nervous system (CNS), since antiretroviral drugs penetrate the CNS poorly. However, as survival improves, the prevalence of HIV encephalopathy (HIVE) continues to rise, and a less fulminant form of HIV-related neurological dysfunction, minor neurocognitive/motor disorder (MCMD), is more commonly seen and remains a significant independent risk factor for AIDS mortality (Ellis et al., 1997; Mattson et al., 2005; McArthur et al., 2005). The brain may also be an important reservoir for the virus, and neurodegeneration and inflammation may progress despite the use of HAART (McArthur et al., 2003; Nath and Sacktor, 2006; Ances and Ellis, 2007). Before the introduction of HAART, most patients with HIVE showed subcortical dementia, with predominant basal ganglia involvement, manifesting as psychomotor slowing, Parkinsonism, behavioral abnormalities and cognitive difficulties (Koutsilieri et al., 2002). Several studies stressed the role of basal ganglia in HIV encephalopathy and suggested that dopaminergic neurotransmission could be impaired in brains of HIV-1 patients, reflecting decreased dopamine (Sardar et al., 1993), and neuronal degeneration in the Substantia Nigra (SN) in brains of AIDS patients (Itoh et al., 2000). Similar findings have been reported in Simian Immunodeficiency Virus (SIV)-infected monkeys (Scheller et al., 2005). HIV-positive patients may show an extreme sensitivity to dopamine receptor antagonists and can develop side effects to antipsychotic agents (Koutsilieri et al., 2002). Neurons themselves are rarely infected by HIV-1, and neuronal damage is felt to be mainly indirect (Kaul et al., 2001). HIV-1 infects periventricular macrophages, resident microglia, and some astrocytes (Ranki et al., 1995), leading to increased production of cytokines, such as TNF-alpha, IL-1beta and IL-6, and chemokines such as MCP-1 (van de Bovenkamp et al., 2002). Macrophages and microglial cells also release HIV-1 proteins, several of which are neurotoxins: envelope (Env) proteins gp41 and gp120, and the nonstructural proteins Tat, Nef, Vpr and Rev. Gp120-induced apoptosis has been demonstrated in studies in cortical cell cultures, in rat hippocampal slices and by intracerebral injections *in vivo* (Meucci et al., 1998). We recently reported that exposure to HIV-1 Env gp120 by injection *in vivo*, induced neuronal apoptosis in the caudate putamen (CP). Gp120-induced apoptosis was prevented by prior local recombinant Simian Virus 40 (rSV40)-based delivery of anti-oxidant enzymes Cu/Zn superoxide dismutase (SOD1) and glutathione peroxidase

* Corresponding author. Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, 1020 Locust Street, Room 255, Philadelphia, PA 19107, USA. Fax: +1 215 503 1269.
E-mail address: jplouboutin@hotmail.com (J.-P. Louboutin).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2009.03.003

(GPx1). These vectors were respectively named SV(SOD1) and SV (GPx1) (Agrawal et al., 2006; Louboutin et al., 2007a). However, tissue loss has also been observed in the brain after direct injection of gp120 (Bansal et al., 2000; Nosheny et al., 2004). Here we document gp120-mediated tissue and neuron loss in the CP as a function of time after injection of gp120 and for different doses of the neurotoxin. We examined the consequences of gp120-induced injury on the dopaminergic pathway in the striatum, dopamine transporter (DAT)-immunoreactivity in the CP, and tyrosine hydroxylase (TH)-immunoreactivity in the SN. Finally, we tested the extent to which prior rSV40 delivery of anti-oxidant enzymes SOD1 and GPx1 to the CP could protect striatal and nigral neurons from gp120-induced injury.

Materials and methods

Animals

Female Sprague–Dawley rats (200–250 g) were purchased from Charles River Laboratories (Wilmington, MA). Protocols for injecting and euthanizing animals were approved by the Thomas Jefferson University Institutional Animal Care and Use Committee (IACUC), and are consistent with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) standards. Because estrogens can regulate microglial activation in some conditions, experiments were done in female rats at similar points of their estrous cycle. The diet that the animals received specifically avoided components that might cause oxidative stress. Numbers of animals used in the experiments are indicated in the "Experimental Design" section.

Antibodies

Different primary antibodies were used: mouse anti-NeuN (1:100) (Chemicon International, Temecula, CA), rabbit anti-human SOD1 (1:100), mouse anti-GPx1 (1:100) (Stressgen, Victoria, B.C., Canada), rabbit anti-DAT (Santa Cruz, Santa Cruz, CA), and mouse anti-TH (Immunostar, Hudson, WI). Secondary antibodies were used at 1:100 dilution: Fluorescein IsoThioCyanate (FITC) and Tetramethyl Rhodamine IsoThioCyanate (TRITC)-conjugated goat anti-mouse, TRITC-conjugated goat anti-rabbit, FITC-conjugated sheep anti-rabbit (Sigma, Saint-Louis, MO), FITC and TRITC-conjugated donkey anti-mouse antibodies (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA).

Vector production

The general principles for making recombinant, *Tag*-deleted, replication-defective SV40 viral vectors have been previously reported (Strayer, 1999). SOD1 and GPx1 transgenes were subcloned into pT7 [RSVLTR], in which transgene expression is controlled by the Rous Sarcoma Virus long terminal repeat (RSV-LTR) as a promoter. The cloned rSV40 genome was excised from its carrier plasmid, gel-purified and recircularized, then transfected into COS-7 cells. These cells supply *in trans* large T-antigen (Tag) and SV40 capsid proteins, which are needed to produce recombinant replication-defective SV40 viral vectors (Strayer et al., 1997). Crude virus stocks were prepared as cell lysates, then band-purified by discontinuous sucrose density gradient ultracentrifugation and titered by quantitative PCR (Q-PCR, Stratagene, Inc.) (Strayer et al., 2001, 2006). SV(human bilirubin-uridine 5′-diphosphate-glucuronosyl-transferase), SV(BUGT), which was used here as a negative control vector, as has been reported (Sauter et al., 2000).

Experimental design

Tissue and neuronal loss study

To test gp120-induced tissue and neuronal loss, different doses of gp120 (100 ng, 250 ng, 500 ng) were injected unilaterally into the CP of the rat brain stereotaxically. For the different doses considered and for all following experiments of the study, gp120 was injected in 1 μl saline. Recombinant HIV-1 BaL gp120 was obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, Germantown, MD. Saline was used as the negative control, as was the contralateral side of the unilaterally injected brains. Brains were harvested 6 h, and 1, 2, 4, 7 and 14 days after the injection and were processed for histological staining (Neutral red-NR, Neurotrace-NT, a marker of neurons) and immunochemistry (for neuN, a neuronal marker, and DAT, the dopamine transporter). The SN of the brains harvested 1, 4, and 7 days after injection of 500 ng gp120 into the CP were immunostained for TH, as were SN sections of brains 7 days after injection with the 3 different gp120 doses. SN sections were also tested by TUNEL assay (Roche, Indianapolis, IN) for assessing the presence of putative apoptotic cells in the SN after injection of 500 ng gp120 into the CP. A total of 72 test rats were used for this study (12 rats for each time point with 4 rats for each dose of gp120 for a particular time point, plus 24 control rats injected with saline, 4 rats at each time point).

Transgene expression study

SV(SOD1) and SV(GPx1) were injected in the CP of 10 rats, whose brains were harvested 4 weeks after injection, 5 rats for each vector. In all cases, controls received saline ($n=2$) or a control SV vector, SV (BUGT) ($n=2$), instead of SV(SOD1) and SV(GPx1) in the CP.

Challenge with gp120

To study the neuroprotection by rSV40-mediated overexpression of SOD1 and GPx1 from gp120-related neuron loss, the CP of rats were first administered with either SV(SOD1) ($n=5$ for each time point considered, total 10 rats) or SV(GPx1) ($n=5$ for each time point studied, total 10 rats). Then, the CP was directly injected with 500 ng gp120 4 weeks after vector injection. Brains were harvested 2 and 7 day after injection of 500 ng gp120 into the CP, and studied for neuronal loss in the CP by NT staining and immunostaining of DAT. Immunoreactivity for TH in the SN was studied 7 days after injection of gp120 into the CP on the same brains as the NT and DAT stainings with 5 rats in each group injected either by SV(SOD1) or SV(GPx1). In all cases, controls received SV(BUGT) instead of SV(SOD1) and SV (GPx1) in the CP ($n=5$ for each time point, total 10 rats).

In vivo transduction and injection of gp120

Rats were anesthetized with isofluorane UPS (Baxter Healthcare Corp., Deerfield, IL) (1.0 U isofluorane/1.5 l O_2 per min) and placed in a stereotaxic apparatus (Stoelting Corp., Wood Dale, IL) for cranial surgery. Body temperature was maintained at 37 °C by using a feedback-controlled heater (Harvard Apparatus, Boston, MA). Glass micropipettes (1.2 mm outer diameter; World Precisions Instruments, Inc., Sarasota, FL) with tip diameters of 15 μm were backfilled with 5 μl of SV(SOD1) or SV(GPx1) viral vector, which contains approximately 10^7 infectious units. The vector-filled micropipettes were placed in the CP using coordinates obtained from the rat brain atlas of Paxinos and Watson (1986). For injection into the CP, a burr hole was placed +0.48 mm anterior to the bregma and −3.0 mm lateral to the sagittal suture. Once centered, the micropipette was placed 6.0 mm ventral from the top of the brain. The same coordinates were used for the injection of gp120 into the CP. The vector was given by a Picospritzer II (General Valve Corp., Fairfield, NJ) pulse of compressed N2 duration 10 ms at 20 psi until the fluid was completely ejected from the pipette. Following surgery, animals were housed individually with free access to water and food.

After a variable survival period, rats were anesthetized via intraperitoneal injection of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) at 60 mg/kg and perfused transcardially though the ascending aorta with 10 ml heparinized saline followed by