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GENE DELIVERY OF ANTIOXIDANT ENZYMES INHIBITS HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 GP120-INDUCED EXPRESSION OF CASPASES

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Abstract—Caspases are implicated in neuronal death in neurodegenerative and other central nervous system (CNS) diseases. In a rat model of human immunodeficiency virus type 1 (HIV-1) associated neurocognitive disorders (HAND), we previously characterized HIV-1 envelope gp120-induced neuronal apoptosis by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. In this model, neuronal apoptosis occurred probably via gp120-induced reactive oxygen species (ROS). Antioxidant gene delivery blunted gp120-related apoptosis. Here, we studied the effect of gp120 on different caspases (3, 6, 8, 9) expression. Caspases production increased in the rat caudate-putamen (CP) 6 h after gp120 injection into the same structure. The expression of caspases peaked by 24 h. Caspases colocalized mainly with neurons. Prior gene delivery of the antioxidant enzymes Cu/Zn superoxide dismutase (SOD1) or glutathione peroxidase (GPx1) into the CP before injecting gp120 there reduced levels of gp120-induced caspases, recapitulating the effect of antioxidant enzymes on gp120-induced apoptosis observed by TUNEL. Thus, HIV-1 gp120 increased caspases expression in the CP. Prior antioxidant enzyme treatment mitigated production of these caspases, probably by reducing ROS levels.

Key words: human immunodeficiency virus type 1, gp120, caspases, apoptosis, antioxidant enzymes, gene therapy.

INTRODUCTION

The caspases family of proteases is conserved from nematodes through mammals. They are central to apoptotic death and are expressed as inactive zymogens that become cleaved during apoptosis (Ribe et al., 2008). Initiator caspases autoactivate and self-process upon recruitment to adaptor proteins. Then, they proceed to cleave and thereby activate the executioner/effector caspases. Activated executioner/effector caspases proceed to process key structural and nuclear proteins and thereby cause the disassembly and death of the cell (Madden and Cotter, 2008). Two major caspases pathways have been described: the intrinsic pathway is initiated by cytochrome c release from the mitochondrion while the extrinsic pathway is initiated by the binding of ligands to plasma-membrane death receptors (Sims and Muyderman, 2010).

Intrinsic apoptosis pathway is required for fetal and postnatal brain development, but is downregulated through the suppression of the expression of one of its key mediators, caspase-3 (Madden and Cotter, 2008). During stroke and neurodegenerative diseases, some caspases are upregulated in the brain (Ribe et al., 2008). Cerebral ischemia triggers both the intrinsic and extrinsic pathways of apoptosis (Broughton et al., 2009; Sims and Muyderman, 2010). Mounting evidence suggests the involvement of caspases in the disease process associated with neurodegenerative diseases such as Alzheimer's disease (AD) (Rohn, 2010) and amyotrophic lateral sclerosis (ALS) (Madden and Cotter, 2008). Caspase activation has also been documented in the brains of patients with human immunodeficiency virus type 1 (HIV-1) associated dementia (Petito and Roberts, 1995; Kaul et al., 2001).

Under physiologic conditions, reactive oxygen species (ROS), which include superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), are generated at low levels and play important roles in signaling and metabolic pathways (Broughton et al., 2009). ROS levels are controlled by endogenous antioxidant such as superoxide dismutases (SOD), glutathione peroxidase (GPx1), glutathione and catalase. Increased levels of ROS are a

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Abbreviations: AD, Alzheimer's disease; AIDS, Acquired Immune Deficiency Syndrome; ALS, amyotrophic lateral sclerosis; APAF-1, apoptotic protease activating factor 1; BUGT, human bilirubin-uridine 5′-diphosphate-glucuronosyl-transferase; CNS, central nervous system; CP, caudate-putamen; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; GFAP, glial fibrillary acidic protein; GPx1, glutathione peroxidase; HAART, highly active anti-retroviral therapeutic drugs; HAD, HIV-1-associated dementia; HAND, HIV-1 associated neurocognitive disorders; H_2O_2, hydrogen peroxide; HIV-1, human immunodeficiency virus type 1; HNE, hydroxynonenal; Iba1, ionized calcium binding adaptor molecule 1; iNOS, inducible nitric oxide synthase; LV, lateral ventricle; MCMD, minor neurocognitive/motor disorder; MDA, malondialdehyde; NO, nitric oxide; NT, neurotrace; OH^-, hydroxyl radical; PD, Parkinson's disease; PBS, phosphate buffer saline; ROS, reactive oxygen species; RSV-LTR, Rous Sarcoma Virus long terminal repeat; SOD1, Cu/Zn superoxide dismutase; SV40, Simian Virus 40; TRITC, tetramethyl rhodamine iso-thiocyanate; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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major cause of tissue injury after cerebral ischemia, in neurodegenerative diseases, as well as in HIV-1 associated neurocognitive disorders (HAND) (Mattson et al., 2005; Antinori et al., 2007; Broughton et al., 2009). Interaction of ROS with other tissue components produces a variety of other radicals: following activation of inducible nitric oxide synthase (iNOS), nitric oxide (NO) can bind superoxide anion to form the highly reactive peroxynitrite (Bonfoco et al., 1995). The latter may attack lipids, proteins and DNA, to enhance oxidant-related injury. Mitochondria are the primary source of ROS involved in many brain tissue injuries (i.e., hypoxia, excitotoxicity). Once generated, mitochondrial ROS influence the release of cytochrome c and other apoptotic proteins from the mitochondria into the neuronal cytosol, which leads to apoptosis (Broughton et al., 2009). For example, once released into the cytosol, cytochrome c forms a complex referred to as an apoptosome with procaspase-9, apoptotic protease activating factor 1 (APAF-1) and dATP. The formation of the apoptosome activates caspase-9 which then cleaves other procaspases. The activation of caspase-3 by this process, among other effectors, has multiple effects including proteolysis of an inhibitor of the caspase-activated DNase (Sims and Muyderman, 2010). Thus, a link between oxidative stress and activation of some caspases seems highly probable.

We previously reported that injection of HIV-1 envelope gp120 into the caudate-putamen (CP) induces neuronal apoptosis, as well as oxidative stress (Agrawal et al., 2006; Louboutin et al., 2007a). We examined here the expression of different caspases, both initiators and effectors, following intra-CP gp120 injection. Finally, we tested if prior gene delivery of the antioxidant enzymes Cu/Zn superoxide dismutase (SOD1) or GPx1 into the CP before injecting gp120 reduces caspases expression.

EXPERIMENTAL PROCEDURES

Animals

Female Sprague–Dawley rats (300–350 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 Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) standards. Experiments were done in female rats at similar points of their estrous cycle determined by vaginal smears. Animals were preferably injected during the diestrus stage of the estrous cycle. Estrogens are typically low during this stage. In any case, animals were not injected during the estrus stage of the cycle when estrogen levels are elevated. The diet that the animals received was a standard commercial, regular powdered rodent diet without any component that might cause oxidative stress (e.g., such as high fat diet, or high manganese) and was not folate/methyl or iron deficient. Animals had free access to water and diet. Numbers of animals used in experiments are indicated in the ''Experimental design'' section.

Antibodies

Diverse primary antibodies were used: rabbit anti-caspase-3 (IgG; 1:100), goat anti-caspase-6 (IgG; 1:100), rabbit anti-caspase-8 (IgG; 1:100), mouse anti-caspase-9 (IgG2a; 1:100) (Santa Cruz, Santa Cruz, CA), rabbit anti-ionized calcium binding adaptor molecule 1 (Iba1) (IgG; 1:100), a marker of quiescent and active microglia (Waco Chemicals, Osaka, Japan), mouse anti-glial fibrillary acidic protein (GFAP) (IgG2b; 1:100) (BD Pharmingen Franklin Lakes, NJ), mouse anti-NeuN (IgG1; 1:100) (Chemicon International, Temecula, CA). Secondary antibodies were used at 1:100 dilution: fluorescein isothiocyanate (FITC) and tetramethyl rhodamine iso-thiocyanate (TRITC)-conjugated goat anti-mouse IgG (γ-chain specific and against whole molecule, respectively), TRITC-conjugated goat anti-rabbit IgG (whole molecule), FITC-conjugated sheep anti-rabbit IgG (whole molecule), FITC-conjugated rabbit anti-goat IgG (whole molecule), Cy3-conjugated rabbit anti-goat IgG (whole molecule) (Sigma, Saint-Louis, MO), FITC and TRITC-conjugated donkey anti-mouse IgG (whole molecule), Cy3-conjugated donkey anti-rabbit IgG (whole molecule) and anti-goat IgG (whole molecule) (Jackson ImmunoResearch Laboratories Inc., WestGrove, PA).

Vector production

The general principles for making recombinant, *Tag*-deleted, replication-defective Simian Virus 40 (SV40) viral vectors have been previously reported (Strayer, 1999; McKee and Strayer, 2002). SOD1 or GPx1 transgenes were subcloned into pT7[RSVLTR], in which transgene expression is driven by the Rous Sarcoma Virus long terminal repeat (RSV-LTR). The cloned rSV40 genome was excised from its carrier plasmid, gel-purified and recircularized, then transfected into COS-7 cells. These cells supply large T-antigen (Tag) and SV40 capsid proteins *in trans*, 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 (Q)-PCR (Strayer et al., 2001). SV(human bilirubin-uridine 5′-diphosphate-glucuronosyltransferase) (BUGT), which was used here as negative control vector, with a non-toxic byproduct, has been reported (Sauter et al., 2000).

Experimental design

Gp120 injection. In order to study gp120-induced abnormalities, 1 μl saline containing 100 ng, 250 ng, or 500 ng gp120 was injected stereotaxically into the CP of rats whose brains were harvested at 6, 24 and 48 h after the injection with 5 rats at each time point; total: $n = 45$ rats). Controls ($n = 4$ at each time point; total: 12) received saline instead of gp120 in the CP. In order to test the specificity of the effects of gp120, 1 μl saline containing 500 ng rat IgG (Sigma) was injected into the CP as a control unrelated protein ($n = 4$ at each time point; total: 12). The contralateral side of the unilaterally injected brains was also used as control. Recombinant HIV-1 BaL gp120 was obtained through the NIH Acquired Immune Deficiency Syndrome (AIDS) Research & Reference Reagent Program, Division of AIDS, NIAID, NIH, Germantown, MD.

Challenge with gp120 after administration of SV(GPx1)/SV(SOD1. To study possible protection by rSV40-mediated overexpression of SOD1 and GPx1 from gp120-related injury, we first injected the CP of rats with SV(SOD1) ($n = 5$) and SV(GPx1) ($n = 5$). One month later, the CP in which SV(SOD1) or SV(GPx1) has been administered was injected with 500 ng gp120. Brains were harvested one day after injection of gp120 into the CP. They were studied for caspases immunoreactivity and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). In all cases, controls received SV(BUGT) in the CP instead of SV(SOD1) and SV(GPx1) ($n = 5$).