Published in final edited form as:

Immunol Invest. 2011 ; 40(5): 481–497. doi:10.3109/08820139.2011.559499.

Methamphetamine and HIV-1 gp120 Effects on Lipopolysaccharide Stimulated Matrix Metalloproteinase-9 Production by Human Monocyte-Derived Macrophages

Jessica L. Reynolds, Supriya D. Mahajan, Ravikumar Aalinkeel, Bindukumar Nair, Donald E. Sykes, and Stanley A. Schwartz

Departments of Medicine, Division of Allergy, Immunology and Rheumatology, State University of New York at Buffalo, Innovation Center, Buffalo, New York 14203

Abstract

Monocytes/macrophages are a primary source of human immunodeficiency virus (HIV-1) in the central nervous system (CNS). Macrophages infected with HIV-1 produce a plethora of factors, including matrix metalloproteinase-9 (MMP-9) that may contribute to the development of HIV-1-associated neurocognitive disorders (HAND). MMP-9 plays a pivotal role in the turnover of the extracellular matrix (ECM) and functions to remodel cellular architecture. We have investigated the role of methamphetamine and HIV-1 gp120 in the regulation of lipopolysaccharide (LPS) induced-MMP-9 production in monocyte-derived macrophages (MDM). Here, we show that LPS-induced MMP-9 gene expression and protein secretion are potentiated by incubation with methamphetamine alone and gp120 alone. Further, concomitant incubation with gp120 and methamphetamine potentiated LPS-induced MMP-9 expression and biological activity in MDM. Collectively methamphetamine and gp120 effects on MMPs may modulate remodeling of the extracellular environment enhancing migration of monocytes/macrophages to the CNS.

Keywords

Methamphetamine; Monocyte-derived mature macrophages; Lipopolysaccharide; Matrix metalloproteinase; gp120; Human immunodeficiency virus

INTRODUCTION

Matrix metalloproteinases (MMPs), a family of zinc- and calcium-dependent endopeptidases, catalyze the proteolysis of the extracellular matrix (ECM) (Kaczmarek et al., 2002; Dzwonek et al., 2004). MMPs are produced by numerous cell types including monocytes, macrophages, lymphocytes, neutrophils, and eosinophils (Visse and Nagasse, 2003). MMPs are synthesized as inactive precursors or proenzymes and are activated following proteolytic removal of the propeptide domain.

MMPs mediate many normal and pathologic biological processes, including cell migration, invasion, proliferation, angiogenesis, embryogenesis and tissue remodeling (Mannello and Gazzanelli, 2001; Van den Steen et al., 2002). MMPs degrade constituents of the ECM contributing to blood-brain barrier (BBB) leakage and infiltration by activated or infected

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Address correspondence to Jessica L. Reynolds, Department of Medicine, Division of Allergy, Immunology and Rheumatology, Innovation Center, 640 Ellicott Street, Buffalo, NY 14203, USA; jlr8@buffalo.edu.

Declaration of interest: The authors alone are responsible for the content and writing of the paper.

immune cells (Mun-Bryce et al., 1998; Vos et al., 2000; Van den Steen et al., 2002). Tissue inhibitors of metalloproteinase (TIMPs) regulate the proteolytic activity of MMPs (Mannello and Gazzanelli 2001). Collectively, MMPs and TIMPs modulate remodeling of the extracellular environment.

Matrix metalloproteinase-9 (MMP-9) is implicated in multiple inflammatory diseases (Van den Steen et al., 2002). MMP-9 is inducible in monocytes/macrophages by tumor necrosis factor (TNF)- α , and lipopolysaccharide (LPS) (Leber and Balkwill, 1998; Van den Steen et al., 2002; Lai et al., 2003; Lu and Wahl, 2005; Rhee et al., 2007). During pathophysiologic conditions, monocytes and macrophages secrete higher levels of MMP-9 (Mun-Bryce and Rosenberg, 1998; Conant et al., 2004; Webster and Crowe, 2006; Westhorpe et al., 2009). Further, MMP-9 is upregulated following insults such as infection, to the brain, suggesting its role in neurodegeneration, neural remodeling and BBB permeability regulation (Conant et al., 1999; Dhawan et al., 1992; Ghorpade et al., 2001; Lee et al., 2003; Louboutin et al., 2010).

Substance abuse is a major public health concern in the United States and several factors contribute to the development and persistence of drug addiction. Methamphetamine is a widely abused illegal drug, which can be snorted, smoked or injected. Worldwide methamphetamine use is estimated at 35 million people, while in the United States it is estimated to be used by more than 10 million people (Hamamoto and Rhodus, 2009). The dual epidemics of drug abuse and human immunodeficiency virus (HIV-1) infection coincide with one another.

HIV-1 enters the central nervous system (CNS) after initial infection (Davis et al., 1992). Monocytes/macrophages are believed to be a primary source of HIV-1 in the CNS via transmigration across the BBB (Wu et al., 2000; Buckner et al., 2006; Eugenin et al., 2006). HIV-1 infections are frequently complicated by a variety of neurological disorders known as HIV-associated neurocognitive disorders (HAND). As many as 50% of HIV-1 infected individuals develop this disorder (Williams et al., 2002). HAND is associated with an increase in the number of activated monocytes within the CNS that secrete viral proteins (Nath et al., 2001; Gendelman et al., 1997; Louboutin et al., 2010).

Moreover, activated macrophages produce a plethora of mediators, including MMPs that presumably contribute to the development of HAND (Louboutin et al., 2010). However the effect of methamphetamine and/or gp120 on MMP-9 expression in macrophages has yet to be elucidated. Therefore, examination of methamphetamine and/or gp120 modulation of LPS-induced MMP-9 in monocyte-derived macrophages (MDM) was investigated. Further, the mechanism(s) of these effects on MMP-9 are unknown therefore a potential mechanism involving p38 MAPK was investigated.

METHODS

Human Subjects

Blood donors were recruited at University at Buffalo; consents were obtained consistent with the policies of University at Buffalo Health Sciences Institutional Review Board (HSIRB) and the National Institutes of Health. Peripheral blood samples from HIV-1 negative individuals were drawn into a syringe containing heparin (20 units/ml, Sigma-Aldrich, St. Louis, MO). A total of 4 independent donors were used.

Isolation of Monocyte Derived Macrophages (MDM)

Human peripheral blood mononuclear cells (PBMC) were separated by Ficoll-Paque (GE Health Care, Piscataway, NJ) gradient centrifugation. CD14⁺ cells were isolated from

PBMC using Dynabeads CD14 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. CD14⁺ cells (1×10^5 cells/well) were cultured in completed medium [RPMI 1640, 10% fetal calf serum, 5% human AB serum, 10 mmol/L HEPES, 1% Penicillin-Streptomycin, 10 ng/ml macrophage colony-stimulating factor (Millipore, Billerica, MA)] for 7 days for differentiation into MDM.

Drug Treatment

Methamphetamine: MDM were pre-treated with 5 μ M methamphetamine hydrochloride (Sigma-Aldrich) in serum free medium for 24 h. The concentration of methamphetamine used was based on previous dose response studies that produced a maximum biological response without causing toxicity to the target cells in previous published in vitro studies (Talloczy et al., 2008). For all experiments, cells treated with vehicle alone (media, referred to as control) were used as the untreated control.

HIV-1 envelope glycoprotein 120 (gp120): MDM were pre-treated with 50 ng/ml HIV-1 gp120 in serum free medium for 24 h. HIV-1 gp120 protein was purified from the macrophage-tropic HIV-1 strain BaL (gp120_{BaL} or R5 gp120) and was obtained from the AIDS Research and Reference Reagent Program, NIAID, NIH. The concentration of gp120 used was based on previous dose response studies (Mahajan et al., 2005).

Lipopolysaccharide (LPS) (isolated from *Escherichia coli* 026:B6; Sigma-Aldrich, cat # L-3755): MDM were pre-incubated with methamphetamine alone, or gp120 alone or in combination for 24 h, washed, media replaced, then stimulated with LPS (100 ng/ml) for 12 h (Serra et al., 2010; Tipton et al., 2010).

Cell Viability Assay and Phagocytosis Assay

MDM (10,000 cells/ml/well) were incubated with methamphetamine (5 μ M) or gp120 (50 ng/ml) for 12, 24, 36 and 48 h.

Cell Viability—MDM were subsequently incubated with the (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT, Sigma-Aldrich) for approximately 3 h, followed by addition of a detergent solution to lyse the cells and solubilize the colored crystals. The samples were read using an ELISA plate reader at a wavelength of 570 nm.

Phagocytosis—Phagocytosis assay was performed according to manufacturer's specifications (Cell Biolabs, San Diego, CA). Briefly, MDM were incubated with zymosan suspension for 2 h. Cells were fixed for 5 min, blocked, then permeabilized with 1X permeabilization solution. Detection reagent was added for 1 h followed by substrate incubation for 20 min. Stop Solution was added and the absorbance of each well was read at a wavelength of 405 nm.

RNA Extraction and Real-Time Quantitative PCR (Q-PCR)

Cytoplasmic RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's specifications. The final RNA pellet was dried and resuspended in diethyl pyrocarbonate water and the concentration of RNA was determined using a *NanoDrop ND-1000* Spectrophotometer (NanoDrop Technologies, Wilmington DE). Any DNA contamination in the RNA preparation was removed by treating the RNA with DNase (1 IU/ μ g of RNA, Promega, Madison WI) for 2 h at 37°C, followed by proteinase K digestion at 37°C for 15 min and subsequent extraction with phenol/chloroform and NH₄OAc/ETOH precipitation.

The isolated RNA was stored at -70°C until used. Relative abundance of each mRNA species was assessed using the SYBR green master mix from Stratagene (La Jolla, CA) to

perform Q-PCR. Differences in threshold cycle number were used to quantify the relative amount of PCR target contained within each tube. Relative mRNA species expression was quantitated and expressed as transcript accumulation index ($TAI = 2^{-(\Delta\Delta CT)}$), calculated using the comparative C_T method. All data were controlled for quantity of RNA input by performing measurements on an endogenous reference gene, β -actin (Shively et al., 2003). All values were normalized to the constitutive expression of the housekeeping gene, β -actin (Mahajan et al., 2005).

ELISA

Total MMP-9 concentrations were measured by ELISA (R&D Systems, Minneapolis, MN). Detection limits were 0.156 ng/mL for MMP-9. Absorbance values were read at 450 nm using a microtiter plate spectrophotometer, and the results are expressed as percent change from control.

Zymography

Cell culture supernatants were collected and centrifuged at 400 g, 5 min. The cell free supernatant was mixed with Novex[®] Tris-Glycine-SDS (2x) sample buffer (Invitrogen). Zymography was performed using precast gels (4–16% Zymogram (Blue Casein) Gels) renaturing buffer and developing buffer, according to the manufacturer's instructions (Invitrogen). Areas of protease activity appeared as clear bands against a dark background (Leber and Balkwill, 1997). Gels analyses were done using a Syngene Image Analyzer with Gene Tools Analysis Software version 3.02.00 (Syngene, Frederick, MD). Data are expressed as percent change from control.

Statistics

Statistical significance was determined using ANOVA followed by Bonferroni post-hoc test (SPSS Inc.).

RESULTS

Cell Viability Assay and Phagocytosis Assay

Methamphetamine (5 μ M) at 12 or 24 h had no effect on cell viability or phagocytosis activity in MDM, (data not shown). This confirms previous published studies (Talloczy et al., 2008; Tipton et al., 2010). gp120 had no effect on cell viability or phagocytosis at 12 or 24 h (Mahajan et al., 2005). However, at 36 and 48 h there was a trend towards a reduction in cell viability and phagocytosis activity (data not shown).

MMP-9 Expression and Activity in MDM

The effects of methamphetamine and gp120 on LPS-induced MMP-9 gene expression were investigated. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12 h. RNA was isolated and gene expression for MMP-9 was determined using Q-PCR. As shown in Figure 1, in the absence of LPS, methamphetamine alone, gp120 alone or concomitant incubation had no significant effect on gene expression for MMP-9. LPS alone significantly increased gene expression for MMP-9 compared to control ($p < 0.001$).

LPS-induced gene expression for MMP-9 was significantly potentiated by pre-incubation with methamphetamine compared to control ($p < 0.001$) and LPS alone ($p < 0.005$). gp120 alone significantly increased LPS-induced gene expression for MMP-9 compared to control ($p < 0.001$) and LPS alone ($p < 0.005$). Pre-incubation with methamphetamine and gp120

concomitantly significantly potentiated LPS-induced gene expression for MMP-9 compared to control ($p < 0.001$) and to LPS alone ($p < 0.005$).

The effects of gp120 and methamphetamine on LPS-induced MMP-9 protein secretion were next investigated using an MMP-9 specific ELISA. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12 h. Supernatants were analyzed for MMP-9 protein secretion. As shown in Figure 2, in the absence of LPS, methamphetamine alone, gp120 alone or concomitant incubation had no significant effect on MMP-9 protein secretion. LPS alone significantly increased protein secretion for MMP-9 compared to control ($p < 0.001$). LPS-induced MMP-9 protein secretion was significantly potentiated by pre-incubation with methamphetamine compared to control ($p < 0.001$) and LPS alone ($p < 0.005$). gp120 alone significantly increased LPS-induced MMP-9 protein secretion compared to control ($p < 0.001$) and LPS alone ($p < 0.005$). Pre-incubation with methamphetamine and gp120 concomitantly significantly potentiated LPS-induced MMP-9 protein secretion compared to control ($p < 0.005$) and to LPS alone ($p < 0.001$).

Biologically active MMP-9 activity was investigated using gelatin zymography. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12 h. Supernatants were assayed for biologically active MMP-9. As shown in Figure 3, in the absence of LPS, methamphetamine alone, gp120 alone or concomitant incubation had no significant effect on biologically active MMP-9. LPS alone significantly potentiated biologically active MMP-9 compared to control ($p < 0.005$). Pre-incubation with methamphetamine alone or gp120 alone, increased MMP-9 expression compared to control ($p < 0.005$, no LPS). Unlike RNA and protein secretion data (ELISA), there was no significant difference between LPS alone and pre-incubation with methamphetamine alone or gp120 alone. However, concomitant incubation with methamphetamine and gp120 potentiated LPS-induced MMP-9 biological activity compared to control ($p < 0.001$) and LPS alone ($p < 0.005$).

p38 MAPK Gene Expression

The effects of gp120 and methamphetamine on p38 MAPK gene expression were investigated. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12 h. RNA was isolated and gene expression for p38 MAPK was determined using Q-PCR. As shown in Figure 4, in the absence of LPS, methamphetamine alone, gp120 alone or concomitant incubation significantly potentiated gene expression for p38 MAPK compared to control ($p < 0.001$). LPS alone significantly increased gene expression for p38 MAPK compared to control ($p < 0.001$). In the presence of LPS, methamphetamine alone, gp120 alone or concomitant incubation significantly potentiated gene expression for p38 MAPK compared to control ($p < 0.001$) and LPS alone ($p < 0.005$).

DISCUSSION

Addictive drug use is connected with high-risk sexual behavior and HIV-1 viral infection. It has been also been suggested to contribute to the development of HAND (Hamamoto and Rhodus, 2001; Conant et al., 2004; Tallozy et al., 2010; Tipton et al., 2010, Nath et al., 2001). Drug abuse and HIV-1 are also associated with an increase in circulating LPS and increased immune activation (Friedman et al., 2003; Ancuta et al., 2008; Brenchley et al., 2006; Brenchley and Douek, 2008). This study sought to investigate the effect that the addictive drug methamphetamine and the HIV-1 viral protein gp120 have on the production of MMP-9 by LPS stimulated macrophage. MMP-9 production has been associated with

disruption of the BBB which may increase the risk for development of HAND (Power et al., 1993; Dallasta et al., 1999; Webster and Crowe, 2006; Louboutin et al., 2010).

Aberrant immune activation associated with HIV-1 disease has been correlated with microbial or microbial by-product translocation into the peripheral blood as a consequence of gastrointestinal tract impairment (Brenchley and Douek, 2008). LPS, a component of the outer membrane of gram-negative bacteria, is an endotoxin that elicits a strong immune response. Brenchley et al. found circulating levels of plasma LPS to be significantly increased in chronically infected HIV-1 patients (Brenchley et al., 2006). Antiretroviral therapy decreased plasma LPS levels (Brenchley et al., 2006; Baroncelli et al., 2009).

Moreover, studies demonstrated an association between a reduction in the levels of plasma LPS and CD4 T-cell reconstitution following antiretroviral therapy (Jiang et al., 2009; Ciccone et al., 2010). Furthermore, subjects with acute and chronic HIV-1 infection had higher levels of plasma soluble CD14 (sCD14, marker of macrophage activation) than HIV-seronegative subjects suggesting a role of activated monocyte/macrophage in the progression of HIV-1 disease (Brenchley et al., 2006).

The incidence and severity of HAND are exacerbated by drugs of abuse (Nath et al., 2001; Ferris et al., 2009). Addictive drug use is associated with an increase in bacterial infections, i.e. increased LPS production (Friedman et al., 2003). Ancuta et al. demonstrated that LPS levels were higher in AIDS patients with IV heroin or ethanol abuse and were lower in patients on HAART, compared to control (Ancuta et al., 2008). This study also found high plasma LPS and LPS-binding protein (LBP) levels, together with low endotoxin core antibody (EndoCAb) levels, were associated with increased soluble markers of monocyte/macrophage activation and HAND in drug users (Ancuta et al., 2008). These authors suggest a role for drug abuse as a cofactor that produces elevated LPS levels in triggering monocyte/macrophage activation thereby contributing to the incidence and severity of HAND.

Peripheral monocytes/macrophages are a primary source of HIV-1 in the CNS and play a central role in the development of HAND. Activated monocytes/macrophages secrete numerous factors including TNF, CCL2, IL-6, and MMP-9 (Herbein and Varin, 2010; Gras and Kaul, 2010). Increased transmigration of HIV-1 infected monocytes, dendritic cells, and leukocytes in an *in vitro* human BBB model has been associated with increased MMP production and a reduction in tight junction proteins and increased BBB permeability (Dhawan et al., 1992; Westhorpe et al., 2009; Louboutin et al., 2010). Patients with HAND have increased serum protein leakage across the BBB (Rhodes, 1991; Annunziata, 2003; Webster and Crowe, 2006; Louboutin et al., 2010).

During pathophysiologic conditions, monocytes and macrophages secrete higher levels of MMP-9 (Mun-Bryce and Rosenberg, 1998; Conant et al., 2004; Webster and Crowe, 2006; Westhorpe et al., 2009). Studies detect MMP-9 in the cerebrospinal fluid (CSF) of HIV-1-infected patients (Sporer et al., 1998; Conant et al., 1999; Liuzzi et al., 2000) and levels are elevated in patients with HIV-associated neurological diseases (Contant et al., 1999; Liuzzi et al., 2000). MMP-9 expression in MDM has been shown to be both upregulated (Dhawan et al., 1992; Kumar et al., 1999) and downregulated (Ghorpade et al., 2001) by HIV-1 infection, depending on the time frame of exposure.

The HIV-1 viral protein gp120 increases the levels of MMP-2 and MMP-9 in the rat brain (Louboutin et al., 2010) and MMP-9 in T cells, glioma cells and astrocytes (Missé et al., 2001; Conant et al., 2004; Ju et al., 2009). Previous studies demonstrate that LPS stimulates MMP-9 expression in monocytes/macrophages (Leber and Balkwill, 1998; Van den Steen et al., 2002; Lai et al., 2003; Lu and Wahl, 2005; Rhee et al., 2007). Since LPS induces MMP-9 production in monocytes/macrophages and addictive drugs are known to increase

LPS levels in HIV-1 patients we sought to investigate the *in vitro* effects of the addictive drug methamphetamine and the HIV-1 envelop glycoprotein gp120 on LPS induced MMP-9 production in MDM. We found that both methamphetamine and gp120 alone potentiate the effects of LPS on MMP-9 expression.

We first examined gene and protein secretion for MMP-9 in MDM. Pre-incubation of MDM with methamphetamine alone or gp120 alone for 24 h prior to stimulation with LPS increased gene expression and protein secretion for MMP-9. Concomitant treatment of MDM with both methamphetamine and gp120 further potentiated the effects of LPS on MMP-9 gene expression and protein secretion. We next examined the biological activity of MMP-9. We found that secreted MMP-9 was biologically active and incubation of MDM with methamphetamine and gp120 concomitantly significantly potentiated LPS-induced MMP-9 biological activity.

Collectively, these experiments demonstrate that methamphetamine and gp120 potentiates the expression and activity of MMP-9 in MDM. Pathologic productions of MMP-9 produced by methamphetamine and gp120 may lead to enhanced tissue remodeling in the CNS by degradation of the ECM. This tissue remodeling may lead to the development of HAND more rapidly in HIV-1 positive subjects, drug users.

The induction of MMP-9 by LPS in monocytes/macrophages has been demonstrated to occur through the phosphatidylinositol-3 kinase (PI-3K), Akt, IKK β , and NF- κ B pathways (Lu and Wahl, 2005; Rhee et al., 2007). Moreover, numerous studies have shown that the mitogen-activated protein kinases (MAPKs) are also involved in the regulation of MMPs by various cell types (Missé et al., 2001; Medders et al., 2010). Missé et al demonstrates that M- and T-tropic gp120 stimulate the secretion of MMP-9 in primary T cells, C6 glioma cells or in CD4⁺/CXCR4⁺ Jurkat T cells through the p38/MAPK pathway (Missé et al., 2001). gp120 activates p38 MAPK in primary neurons and microglial cells (Kaul et al., 2007).

Activation of macrophages by gp120 has been suggested to be regulated by PI3K, Akt, and MAPK proteins (Kaule et al., 1999; Kaul et al., 2007; Perfettini et al., 2005; Cheung et al., 2008; Medders et al., 2010). Medders et al., demonstrate that monocyte/macrophage-like THP-1 cells and MDM require p38 MAPK for gp120 neurotoxin production (Medders et al., 2010). Our studies demonstrate that methamphetamine, gp120 or concomitant incubation increase gene expression for p38 MAPK in MDM. Although these findings are significant, the increased expression of p38-MAPK does not necessarily correspond to increased activation of this pathway. We can only speculate, based on our data and previous published studies (Medders et al., 2010), that p38 MAPK plays a role in regulating methamphetamine and gp120 induced MMP-9 expression in MDM. Further studies are needed to elucidate the role of p38 MAPK in methamphetamine and gp120 potentiation of LPS-stimulated MMP-9 production in MDM.

Previous studies demonstrate catecholamines potentiate LPS-induced effects on MMP-1 and MMP-9 and activity in U937 cells (Speidl et al., 2004). The current study suggests the *in vivo* effects of methamphetamine may be due to its effects on neurotransmitters, in particular dopamine. Repeated methamphetamine treatment induces behavioral sensitization, and increases in MMP-2/-9/TIMP-2 activity in the brain. Furthermore, MMP-2/-9 inhibitors block methamphetamine-induced behavioral sensitization and reduce methamphetamine-increased dopamine release in the NAc (Mizoguchi et al., 2007a & b).

These data suggest that methamphetamine *in vivo* modulates dopamine to regulate MMP-9 expression. An elegant study by Gaskill et al., demonstrates the MDM express dopamine receptors 1 and 2, and dopamine enhances HIV-1 replication in MDM (Gaskill et al., 2009). Furthermore, studies in SK-N-MC human neuroblastoma cells demonstrate that an agonist

to the D₁ dopamine receptor induced similar time- and dose-related activation of p38 MAPK and c-Jun amino-terminal kinase (JNK) (Zhen et al., 1998). These data suggest a potential *in vivo* role of dopamine in the regulation of MMPs. Collectively, these data demonstrate the potentially deleterious effects of methamphetamine on the HIV-1 epidemic and a need to further understand the effects of methamphetamine on the immune system.

Acknowledgments

Funding support: K01 DA024577 (Reynolds); R01AI085569 (Schwartz); R21DA030108 (Mahajan); Kaleida Health Foundation (Schwartz).

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Figure 1.

The effect of methamphetamine and/or gp120 on LPS-stimulated MMP-9 gene expression from MDM. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12 h. RNA was isolated and gene expression for MMP-9 was determined using Q-PCR. Meth = methamphetamine. Statistical significance was calculated using ANOVA followed by Bonferroni post-hoc test, *compared to control ($p < 0.005$); †compared to LPS alone ($p < 0.001$). Data represent the mean $\pm$ standard deviation, $n = 4$.

Figure 2.

The effect of methamphetamine and/or gp120 on LPS-stimulated MMP-9 secretion from MDM. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12 h. Supernatants were collected and analyzed with an MMP-9 ELISA. Meth = methamphetamine. Statistical significance was calculated using ANOVA followed by Bonferroni post-hoc test, *compared to control ($p < 0.005$); †compared to LPS alone ($p < 0.001$). Data represent the mean $\pm$ standard deviation, $n = 4$.

Figure 3.

The effect of gp120 and methamphetamine on LPS-stimulated biologically active MMP-9 secreted from MDM. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12. Supernatants were collected and analyzed for biologically active MMP-9 using zymography. Meth = methamphetamine. A. Representative zymograph. B. Gels analyses were done using a Syngene Image Analyzer with Gene Tools Analysis Software version 3.02.00. Statistical significance was calculated using ANOVA followed by Bonferroni post-hoc test, * compared to control ($p < 0.005$); † compared to LPS alone ($p < 0.001$). Data represent the mean $\pm$ standard deviation, $n = 4$.

Figure 4. p38 MAPK kinase expression. MDM were incubated with methamphetamine alone (5 μ M), gp120 alone (50 ng/ml) or concomitantly for 24 h, MDM were washed then stimulated with LPS (100 ng/ml) for 12 h. RNA was isolated and gene expression for p38 MAPK was determined using Q-PCR. Meth = methamphetamine. Statistical significance was calculated using ANOVA followed by Bonferroni post-hoc test, * compared to control ($p < 0.005$); † compared to LPS alone ($p < 0.001$). Data represent the mean $\pm$ standard deviation, $n = 4$.