Methamphetamine Facilitates HIV Infection of Human Monocytes Through Inhibiting Cellular Viral Restriction Factors

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Research Article

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Abstract

Background

Methamphetamine (METH), a potent addictive psychostimulant, is highly prevalent in HIV-infected individuals. Clinically, METH use is implicated in alteration of immune system and increase of HIV spread/replication. Therefore, it is of importance to examine whether METH has direct effect on HIV infection of monocytes, the major target and reservoir cells for the virus.

Result

METH-treated monocytes were more susceptible to HIV infection as evidenced by increased levels of viral p24 protein and expression of viral GAG gene. Mechanistically, METH treatment of monocytes inhibited the expression of the antiviral IFN-stimulated genes (ISGs: OAS2, GBP5, ISG56, Viperin and ISG15) and the HIV restriction microRNAs. In addition, METH treatment of monocytes significantly decreased STAT1 expression at both mRNA and protein levels.

Conclusions

These findings suggest a previously unrecognized mechanism for HIV persistent infection in the primary target and reservoir cells.

Introduction

METH is one of the most widely abused illicit drugs among HIV-infected individuals. METH use and HIV infection frequently coexist due to the association of METH use with engagement of high-risk behaviors [1–3]. There is a high prevalence of HIV infection in METH using population [4, 5]. Among men who have sex with men, those who use METH are more susceptible to HIV infection than non-users [6–10]. Clinically, METH use has been implicated in HIV disease progression [11]. Active METH users with HIV infection display higher levels of viral load than non-users [12]. In addition, METH users have delayed viral suppression after initiation of antiretroviral therapy (ART), higher levels of blood HIV RNA, increased frequency of drug resistance mutations and accelerated progression to AIDS [13–17]. METH abuse contributes to CD4⁺ T cells depletion, inflammation/immune activation, and the promotion of HIV entry and disease progression [18].

Cells of monocyte/macrophage lineage are crucial in initial HIV infection and implicated in the immunopathogenesis of HIV disease. Monocytes are among the first and major cell types infected by HIV and function as reservoirs for the virus. However, unlike tissue macrophages and *in vitro* monocyte-derived macrophages which are highly susceptible to HIV infection, peripheral blood monocytes are refractory to HIV infection *in vivo* and *in vitro*, and only a small percentage of monocytes harbor the virus

[19, 20]. Despite of their resistance to HIV infection, monocytes are involved in HIV infection of the central nervous system (CNS) as they can bring the virus to the brain [21]. A study reported that HIV-infected monocytes are more likely to cross the blood brain barrier (BBB) as compared to uninfected monocytes [22]. Among HIV-infected METH users, HIV-associated neurocognitive disorders (HAND) are the more frequent and severe [23, 24]. A recent study demonstrated that METH could enhance HIV infection of neural progenitor cells, a possible mechanism for the impairment or disruption of neurocognitive functioning in HIV-infected individuals with NeuroAIDS [25]. Several studies showed that elevated extracellular CNS dopamine by METH abuse could facilitate uninfected and HIV-infected CD14⁺CD16⁺ monocytes transmigration across the BBB, resulting in the propagation of viral reservoirs and inflammation in the CNS which contribute to the development of HAND [26, 27]. Thus, it is of a great interest to determine the impact of METH on susceptibility of peripheral blood monocytes to HIV infection. In addition, it is critical to understand the pathological effects of METH on the specific intracellular innate immunity against HIV in monocytes.

Results

METH enhances HIV infection

We first examined cytotoxicity effect of METH on monocytes. As shown in Supplemental Fig. 1, METH at the concentration as high as 1000 μ M had little effect on cell viability. We then studied whether METH could enhance susceptibility of monocytes to HIV infection. As demonstrated in Figs. 1A and 1B, METH treatment of monocytes significantly increased the expression of both intracellular and extracellular HIV GAG gene expression. In addition, METH-treated monocytes showed higher levels of HIV p24 protein than untreated cells (Figs. 1C and 1D). The enhancing effects of METH on HIV GAG gene expression and p24 protein production were dose-dependent (Figs. 1A-1D).

METH suppresses antiviral ISGs

We next examined the effect of METH on the expression of the intracellular antiviral ISGs. As shown in Figs. 2A and 2B, METH dose-dependently inhibited the expression of the antiviral ISGs (OAS2, GBP5, ISG56, Viperin, ISG15) at 24 h post-treatment. In addition, the Western blot analysis demonstrated that METH-treated monocytes had lower protein levels of the antiviral ISGs than the untreated cells (Fig. 2C). To determine whether the inhibitory effect of METH on the ISGs is not associated with cytotoxicity, we treated monocytes with different concentrations of METH for 4 days. We found that METH at the concentration as high as 1000 μ M had little toxic effect on monocytes (Supplementary Fig 1).

METH inhibits IRF7 and STAT1

To study the mechanisms of METH-mediated ISGs inhibition, we examined whether the expression of several IFN regulatory factors (IRFs), the key modulators of the IFNs/ISGs signaling pathway, are affected by METH. As shown in Fig. 2D, although METH had little effect on the expression of IRF1, IRF3 and IRF5, METH-treated cells expressed lower levels of IRF7 at both mRNA and protein levels as compared to the

untreated cells. We also examined whether METH has a negative impact on the expression of STAT family members including STAT1, STAT2 and STAT3, which are crucial in the host innate immunity against viral infection [28, 29]. As shown in Fig. 2E, while METH treatment of monocytes had little effect on STAT2 and STAT3 expression, it significantly inhibited STAT1 expression at both mRNA and protein levels.

METH inhibits HIV restriction miRNAs

Our earlier study showed that monocytes contain significantly higher levels of the HIV restriction miRNAs than monocyte-derived macrophages, which explains why monocytes are refractory to HIV infection [30]. We thus investigated whether METH negatively influences the expression of the HIV restriction miRNAs in monocytes. As shown in Fig. 2F, METH treatment of monocytes suppressed the expression of the intracellular HIV restriction miRNAs (mi-28, miR-29 family members, miR-125b, miR-150, miR-223, and miR-382). In addition, we observed that lower levels of these HIV restriction miRNAs in supernatant of monocyte cultures 36 h after METH treatment than those in untreated cells (Fig. 2G).

Discussion

Although METH use has been linked to HIV transmission and infection, its pathological effects on the host cell-mediated specific innate immunity against HIV infection remain to be determined. The earlier studies reported that METH could enhance HIV infection of several cell types, including dendritic cells [31], macrophages [32, 33], CD4⁺ T cells [34, 35], and neural progenitor cells [25]. However, it is unclear whether METH facilitates HIV infection of primary human monocytes. In the present study, we demonstrated that METH treatment of the monocytes significantly enhanced HIV infection/replication at both intracellular and extracellular levels (Figs. 1A-1D). To investigate the underlying mechanisms of METH-mediated HIV enhancement in monocytes, we examined that the impact of METH on the expression of IFNs and the downstream effectors in IFNs signaling pathway. We found that although METH treatment of monocytes had little effect on IFN- α and IFN- β expression (data not shown), it down-regulated the expression of the antiviral ISGs, including OAS2, GBP5, ISG56, Viperin, and ISG15 (Figs. 2A-2C). These ISGs are known to have the ability to restrict HIV replication at different steps of viral replication cycle [36–40]. For instance, Krapp et al demonstrated that the expression of GBP5 could interfere with the processing and virion incorporation of the HIV envelope glycoprotein, which remarkably reduce virion incorporation of mature gp120 and enhance virion-associated immature gp160 precursor, leading to the inhibition of HIV infectivity [37]. Okumura et al showed that ISG15 impaired the interaction between HIV GAG and tumor susceptibility gene 101 (Tsg101), and suppressed HIV virion release [41].

Given that the IFN signaling pathway is responsible for the production of the antiviral ISGs, we also examined the impact of METH on IFN regulatory factors (IRFs), particularly IRF1, IRF3, IRF5 and IRF7, the key players in regulating the expression of antiviral ISGs and producing an antiviral state [42]. We found that while METH had little effect on IRF1, IRF3, IRF5, it specifically suppressed IRF7 expression in monocytes at both mRNA and protein levels (Fig. 2D). In addition, we observed that METH treatment of

monocytes inhibited the expression of STAT1, a crucial regulatory factor in IFNs-mediated induction of antiviral ISGs [43, 44]. The effect of METH on STAT1 appeared to be specific, as it had little effect on the expression of STAT2 and STAT3 (Fig. 2E).

In addition to the HIV restriction factors of protein nature, a cluster of HIV restriction miRNAs have also been revealed to contribute to HIV latency in resting CD4⁺ T cells [45, 46]. These cellular miRNAs interact with the 3'-termini of HIV RNA, resulting in the transcriptional inefficiency and post-transcriptional suppression [47, 48]. We documented that monocytes expression much higher levels of HIV restriction miRNAs (miRNA-382, -223, -150, and -28) than monocyte-derived macrophages, which may contribute to HIV latency in monocytes [30]. Therefore, it is of interest to determine whether METH use inhibits these HIV restriction miRNAs, which are implicated in HIV latency. Among these cellular miRNAs, the miR-29 family members (miR-29a, miR-29b, and miR-29c) suppress HIV replication by targeting a highly conserved region of HIV [49]. Importantly, our early study documented that the levels of the cellular HIV restriction miRNAs negatively correlated with susceptibility of monocytes and macrophages to HIV infection [30]. We also reported that ART failed to restore the levels of several HIV restriction miRNAs in PBMCs of HIV-infected men who have sex with men who used METH [50]. Therefore, it is clinically relevant to seek the direct evidence of how METH negatively impacts on the HIV restriction miRNAs. Our observation that METH could significantly suppress the expression of the HIV restriction miRNAs (Figs. 2F and 2G) provide not only direct evidence for our *in vivo* observation [50], but also an additional mechanism for METH-mediated enhancement of HIV infection.

In summary, we demonstrate that METH can enhance HIV infection in monocytes through the inhibition of the multiple cellular antiviral factors (ISGs and miRNAs) at both mRNA and protein levels. Although it is possible that there are additional mechanisms involved in the METH action on HIV, compromising the intracellular immunity that is crucial for control of HIV replication should be responsible for much of METH-mediated HIV enhancement in monocytes. These findings suggest that METH use is a contributing factor for HIV infection and persistence in monocytes. As HIV-infected individuals are living longer with ART and many of infected individuals are METH users, to further identify the pathological role of METH in HIV-infected reservoir cells is necessary for understanding the mechanisms of HIV persistence and developing strategies for the viral eradication.

Conclusions

These findings suggest a previously unrecognized mechanism for HIV persistent infection in the primary target and reservoir cells.

Materials And Methods

Cells and reagents

Purified human peripheral blood monocytes were obtained from Human Immunology Core at the University of Pennsylvania (Philadelphia, PA, USA). The Core has the Institutional Review Board approval for blood collection from healthy donors. Freshly isolated monocytes were cultured in 1640 RPMI medium supplemented with 10% fetal bovine serum, 1% nonessential amino acid, 1% L-glutamine and 1% penicillin-streptomycin solution. Rabbit antibodies against OAS2, GBP5, ISG56, Viperin, ISG15, IRF7, p-IRF7, STAT1, p-STAT1 and GAPDH were purchased from Cell Signaling Technology (Danvers, MA, USA). Mouse anti-HIV p24 antibody was obtained from the NIH AIDS Research and Reference Reagent Program (Bethesda, MD, USA). METH was purchased from Sigma Aldrich (St Louis, MO, USA). METH powder was dissolved in sterile endotoxin-free water (HyPure™ Cell culture grade water, GE Healthcare Life Science, Logan, UT, USA) and stored at 4°C.

HIV infection and METH Treatment

HIV Bal strain was obtained from AIDS Reagent Program (NIH, Bethesda, MD). Freshly isolated and purified monocytes in a 48-well plate were treated with METH at clinically relevant concentrations [51-54] (0, 100, 150, and 250 µM) for 24 h before being infected with HIV Bal strain (p24 60 ng/10⁶ cells) overnight. The cells were then washed three times with plain RPMI to remove any unabsorbed virus and cultured in the presence of METH for 72 h.

MTS assay

The cytotoxic effect of METH on monocytes was evaluated by MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, innersalt) assay. Freshly isolated human blood monocytes (2 × 10⁴ cells/well) were placed in 96-well round bottom plates, and treated with different concentrations of METH (0, 100, 150, 250, 400, 600, and 1000 µM) for 96h. The cells were then incubated with CellTiter 96® AQ_{ueous} One Solution Reagent (Promega Corporation, Madison, WI) containing MTS and phenazine ethosulfate for 4 h at 37°C. Absorbance at 490 nm was measured by a plate reader (SpectraMax i3, Molecular Devices, Sunnyvale, CA, USA).

ELISA and western blot assays

HIV p24 protein levels in monocyte culture supernatant were determined by HIV p24 ELISA kit from Abnova (Taipei, Taiwan) as instructed by the manufacturer. HIV p24 protein levels in uninfected and infected monocytes were determined by Western blot assay. Briefly, the cell pellets were lysed with RIPA lysis buffer supplemented with protease/phosphatase inhibitors (Sigma Aldrich, St Louis, MO). The protein concentrations were determined by the bicinchoninic acid (BCA) assay (ChemCruz, Dallas, TX). The blots were incubated with primary antibodies in 5% nonfat milk in PBS overnight at 4°C, then washed with PBS containing 0.5% Tween. The blots were further incubated with horseradish peroxidase-conjugated second antibodies at room temperature for an hour, then washed with PBST and visualized by enhanced chemiluminescence (Amersham, Bucks, UK) in a Fuji Film LAS-4000 imaging analyzer (GE Life Sciences, NJ, USA).

RNA and microRNA extraction and quantification

Freshly isolated monocytes in 48-well plates were treated with or without METH (100, 150, and 250 μ M) for different time points (0, 6, 12, and 24 h). Total RNAs were extracted with Tri-reagent (Molecular Research Center, OH, USA). RNA (1 μ g) was subjected to reverse transcriptase PCR using reagents from Promega (Promega, WI, USA). The cDNA sample was then subjected to the real-time PCR using iQ SYBR Green Supermix (Bio-Rad Laboratories, CA, USA). All values were normalized to GAPDH mRNA. The sequences of oligonucleotide primers used in this study are listed in Table 1. Extracellular miRNAs were extracted from supernatant of monocyte cultures using the miRNeasy Mini Kit (Qiagen, CA, USA). The miRNAs from cells or supernatant were reversely transcribed with miScript Reverse Transcription Kit (Qiagen, CA, USA). The real-time PCR for the miRNAs quantification was carried out with miScript Primer Assays using miScript SYBR Green PCR Kit from Qiagen as previously described [30].

HIV GAG gene quantification

HIV GAG gene copy numbers in monocytes or monocytes culture supernatant were determined by the real-time PCR. RNAs from cells or the cell-free supernatant were extracted with Tri-reagent (for tissues, cells cultured in monolayer, or cell pellets) or Tri-reagent (for whole blood, serum/plasma or cell culture supernatant) according to the manufacturer's instructions, respectively. HIV GAG standards with known copy numbers were used to quantify viral GAG gene expression in the culture supernatant.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD) of three experiments using monocytes from three different donors. Statistical significance was measured by Student's t-test using GraphPad Prism Statistical Software (GraphPad Software, La Jolla, USA). * P < 0.05 and ** P < 0.01 indicate statistic difference between compared groups.

Declarations

Author Contributions

YL, FZM, XW, WZH designed the research; YL, FZM, PW, JBL, and WHH performed the experiments and analyzed the data; YL, FZM, XW, and WZH interpreted the data and wrote the paper; YL, FZM, XW, JBL, WHH, WZH discussed and edited the paper.

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Not applicable

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Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish this paper.

Competing interests

No potential conflict of interest was reported by the authors.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1
Primers Pairs

Gene	Orientation	Sequence (5'-3')
GAPDH	Forward	GGTGGTCTCCTCTGACTTCAACA
	Reverse	GTTGCTGTAGCCAAATTCGTTGT
HIV GAG	Forward	ATAATCCACCTATCCCAGTAGGAGAAA
	Reverse	TTTGGTCCTTGTCTTATGTCCAGAATGC
OAS2	Forward	CAGTCCTGGTGAGTTTGCAGT
	Reverse	ACAGCGAGGGTAAATCCTTGA
GBP5	Forward	CAGGAACAACAGATGCAGGA
	Reverse	TCATCGTTATTAACAGTCCTCTGG
ISG56	Forward	TTCGGAGAAAGGCATTAGA
	Reverse	TCCAGGGCTTCATTCATAT
Viperin	Forward	TGGGTGCTTACACCTGCTG
	Reverse	TGAAGTGATAGTTGACGCTGGT
ISG15	Forward	GGCTGGGAGCTGACGGTGAAG
	Reverse	GCTCCGCCCCGCCAGGCTCTGT
	Reverse	TCAGTCTTGTTTTCAACGTGGT
IRF1	Forward	TGAAGCTACAACAGATGAGG
	Reverse	AGTAGGTACCCCTTCCCATC
IRF3	Forward	ACCAGCCGTGGACCAAGAG
	Reverse	TACCAAGGCCCTGAGGCAC
IRF5	Forward	AAGCCGATCCGGCCAA
	Reverse	GGAAGTCCCGGCTCTTGTTAA
IRF7	Forward	TGGTCCTGGTGAAGCTGGAA
	Reverse	GATGTCGTCATAGAGGCTGTTGG
STAT1	Forward	CCGTGGCACTGCATACAATC
	Reverse	ACCATGCCGAATTCCCAAAG
STAT2	Forward	CCCCATCGACCCCTCATC
	Reverse	GAGTCTCACCAGCAGCCTTGT

Gene	Orientation	Sequence (5'-3')
STAT3	Forward	CTGCCCCATACCTGAAGACC
	Reverse	TCCTCACATGGGGGAGGTAG

Figures

Figure 1

METH enhances HIV infection in vitro. Freshly isolated human monocytes were treated with METH for 24 h and then infected with HIV Bal strain overnight, washed with PBS three times and cultured in the presence of METH for 72 h. RNAs from cells (A) and the cell-free supernatant (B) were extracted and subjected to the real-time PCR using HIV GAG gene primers. (C) Proteins extracted from cells were subjected to Western blot using the antibodies against p24 and GAPDH. (D) The cell-free supernatant

was subjected to ELISA assay to determine p24 protein level. Data shown were the mean $\pm$ SD from three independent experiments with monocytes from three different donors (* $P < 0.05$, ** $P < 0.01$).

Figure 2

METH inhibits viral restriction factors expression. Freshly isolated monocytes were treated with METH (150 μ M) for the indicated times (A) or at the indicated concentrations for 24 h (B). The cellular RNAs were extracted and subjected to the real-time PCR. (C) Monocytes were treated with METH (150 μ M) for

the indicated times, the cellular proteins were then collected and subjected to Western blot assay. (D and E) The cellular RNAs were extracted and subjected to the real-time PCR to determine the mRNA levels of IRFs and STATs. The cellular proteins were extracted and analyzed with the antibodies against IRF7, p-IRF7, STAT1, p-STAT1 and GAPDH. (F) Monocytes were treated with METH at the indicated concentrations for 6 h. Cellular miRNAs were quantified by the real-time PCR. RNU48 was set as a control gene. (G) Monocytes were treated with METH (150 μ M) for the indicated times. MiRNAs in the culture supernatant were quantified by the real-time PCR. Synthetic *caenorhabditis elegans* miRNA-39 (cel-miR-39) was used as a spiked-in control miRNA for normalization. Data shown were obtained as the mean $\pm$ SD from three independent experiments with monocytes from three different donors (* P <0.05, ** P < 0.01).

Supplementary Files

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