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Independent and Co-morbid HIV Infection and Meth Use Disorders on Oxidative Stress Markers in the Cerebrospinal Fluid and Depressive Symptoms

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Abstract Both HIV infection and Methamphetamine (Meth) use disorders are associated with greater depressive symptoms and oxidative stress; whether the two conditions would show additive or interactive effects on the severity of depressive symptoms, and whether this is related to the level of oxidative stress in the CNS is unknown. 123 participants were evaluated, which included 41 HIV-seronegative subjects without substance use disorders (Control), 25 with recent (<6 months) moderate to severe Meth use disorders (Meth), 34 HIV-seropositive subjects without substance use disorders (HIV) and 23 HIV+Meth subjects. Depressive symptoms were assessed with the Center for Epidemiologic Studies-Depression Scale (CES-D), and oxidative stress markers were evaluated with glutathione (GSH), 4-hydroxynonenal (HNE), and activities of gamma-glutamyltransferase (GGT) and glutathione peroxidase (GPx) in the cerebrospinal fluid (CSF). Compared with Controls, HIV subjects had higher levels of HNE (+350 %) and GGT (+27 %), and lower level of GSH (−34 %), while Meth users had higher levels of GPx activity (+23 %) and GSH (+30 %). GGT correlated with GPx, and with age, across all subjects ($p<0.0001$). CES-D scores correlated with CSF HNE levels only in Control and HIV groups, but not in Meth and HIV+Meth groups. HIV and Meth use had an interactive effects on depressive symptoms, but did not

show additive or interactive effects on oxidative stress. The differential relationship between depressive symptoms and oxidative stress response amongst the four groups suggest that depressive symptoms in these groups are mediated through different mechanisms which are not always related to oxidative stress.

Keywords Depression · HIV · Methamphetamine · Glutathione · Antioxidant · Oxidative stress

Introduction

More than one third of HIV-infected individuals have major depression (Bing et al. 2001), which is 4–5 folds higher than the prevalence of 6.7 % among adults in the United States (Kessler et al. 2005). The somatic symptoms of depression are associated with accelerated disease progression and shortened survival in HIV patients (Farinpour et al. 2003). Depression is also common in individuals who use methamphetamine (Meth) (Zweben et al. 2004), a drug associated with HIV risk behaviors (Cartier et al. 2008). One study showed that the concurrence of Meth use disorders and HIV infection resulted in greater depressive symptoms than either factor alone (Bousman et al. 2009). Since higher levels of lipid peroxidation and lower levels of antioxidants were commonly detected in patients with depression (Maes et al. 2000, 2010), HIV infection (Eck et al. 1989; Aukrust et al. 2003; Awodele et al. 2012), and those with Meth use disorders (Silverstein et al. 2011), one may speculate that the concurrence of HIV infection and Meth use disorders might further elevate oxidative stress in the brain, as shown in animal studies (Flora et al. 2003; Banerjee et al. 2010), which may contribute to the greater depressive symptoms.

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However, HIV infection and Meth use disorders seem to differentially regulate the metabolism of glutathione (GSH), which is the most abundant endogenous antioxidant in the brain, and a substrate for the antioxidant enzymes glutathione peroxidase (GPx) and glutathione-S-transferase (GST) (metabolic pathway shown in Fig. 1). HIV patients had lower GSH levels in plasma and lymphocytes (Eck et al. 1989; Aukrust et al. 2003; Awodele et al. 2012). In contrast, Meth users showed a compensatory response with elevated levels of oxidized GSH (GSSG) in the caudate, but normal levels of GSH in the cortex, hippocampus, and white matter (Mirecki et al. 2004). Animal studies also indicated that acute Meth exposure could temporarily upregulate GSH concentration in the brain (Harold et al. 2000). However, whether the concurrence of HIV infection and Meth use disorders would show additive or interactive effects on oxidative stress and GSH metabolism in the CNS is unknown.

We hypothesized that HIV infection and Meth use disorders have additive effects on brain GSH metabolism; since GSH has a pivotal role in the antioxidant system (Gu et al. 2015), alterations in GSH metabolism affect the oxidative stress level in the CNS, which in turn would affect the severity of depressive symptoms, levels of cognitive function, and general well being. To test this hypothesis, we recruited HIV-seropositive and

HIV-seronegative subjects, with or without Meth use disorders, and assessed the depressive symptoms (using the Center for Epidemiologic Studies - Depression Scale, CES-D), cognitive function (HIV dementia scale), and overall well being (Karnofsky scale) in each subject, and measured various markers of GSH metabolism and oxidative stress in the cerebrospinal fluid (CSF), including the concentrations of GSH and 4-hydroxynonenal (HNE), and the activities of gamma-glutamyl transferase (GGT) and glutathione peroxidase (GPx).

Materials and Methods

Research Participants

All participants were recruited from the local community through flyers and word-of-mouth. From 1,881 individuals screened by telephone, 473 were evaluated by a physician, 282 that fulfilled study criteria were enrolled, and 123 consented to lumbar punctures for this study. These subjects included HIV-positive subjects without a history of substance use disorders (HIV, $n=34$), HIV-positive subjects with Meth use disorder, (HIV+Meth, $n=23$); HIV-seronegative subjects without a history of substance use disorders (Control, $n=41$), and HIV-seronegative with Meth use disorder, (Meth, $n=25$). Each subject provided verbal and written consent for the study, which was approved by the Institutional Review Board (IRB) of the University of Hawaii.

The inclusion criteria were: men or women older than 18 years and able to provide informed consent. Subjects in the HIV and HIV+Meth groups were seropositive for HIV, had nadir CD4 counts <500 cells/mL, were stable on combination antiretroviral therapy (cART) for at least 6 months or not receiving cART, and did not have severe HIV-associated dementia (to ensure they could provide consent). Subjects in the Meth and HIV+Meth groups were within 6 months of abstinence from Meth use, but all met the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria for a history of moderate or severe Meth use disorders; additional inclusion criteria were the use of at least 0.5 g/day of Meth, and use at least three times a week for more than 2 years. Subjects in the Meth and Control groups were seronegative for HIV. Subjects were excluded if they had any confounding neuropsychiatric disorders (except for mild depressive symptoms), significant renal or hepatic dysfunction, any abnormal blood tests (blood count, platelets, and liver function tests) that might indicate a coagulopathy or any other contraindications for the lumbar puncture. They were also excluded if they had a positive urine toxicology (including Meth, cocaine, opiates, barbiturates and benzodiazepines) on the day of the lumbar punctures, or a history of drug use disorders, except for Meth use in the Meth user groups and tobacco cigarette or marijuana smoking in all subjects. Each subject was examined by a

Fig. 1 Glutathione metabolism pathway in the cytoplasm and in extracellular space. *Black arrows*, glutathione catabolism; *green arrows*, antioxidant function of glutathione; *red arrows*, glutathione *de novo* synthesis. * Rate-limiting components. *Abbreviation*: Cys cysteine; DPEP dipeptidase; EAAC1 excitatory amino acid carrier 1; EAAT excitatory amino acid transporters; GCS glutamylcysteine synthetase; GGT glutamyl transferase; Glu glutamate; Gly glycine; GPx glutathione peroxidase; GS glutathione synthetase; GSH glutathione; GSSG oxidized glutathione; HNE 4-hydroxynonenal; HNE-GSH 4-hydroxynonenal-glutathione adduct; MRP multidrug resistance proteins

physician and had a detailed structured neuropsychiatric assessment, including their depressive symptoms (CES-D) (Radloff 1977) and any past or current substances used (including tobacco, alcohol and marijuana). The HIV subjects were also assessed for their HIV disease severity (nadir and current CD4 counts, viral load, duration of HIV infection, HIV dementia scale and Karnofsky scale), while the Meth users were assessed for detailed Meth usage (amount per use, recency of use, age of first use and years of use).

CSF Sample Collection and Processing

CSF samples were collected via lumbar puncture using a 22-gauge atraumatic Sprotte® needle, with the subjects in a sitting position and the puncture sites anesthetized with 2 % lidocaine without epinephrine. The samples were kept on ice and quickly centrifuged at 400×g and 4 °C for 10 min to remove cells if present. The supernatant of each sample was transferred into fresh polypropylene micro-centrifuge tubes and further centrifuged at 1000×g and 4 °C for 10 min to remove any cellular debris. The final supernatant was aliquoted and stored at −80 °C.

Chemicals and Instruments

All chemicals used in this study were purchased from Sigma (St. Louis, MO) unless otherwise noted. The concentration tubes (cut-off size=3 kDa) were purchased from Millipore (Amicon Ultra-0.5, Billerica, MA). The spectrophotometers used were SpectraMax 340 (for GSH, HNE-His, and TBARS assays) and SpectraMax M3 (for GGT and GPx assays) from Molecular Devices (Sunnyvale, CA).

GSH Assay (Pang et al. 2013)

The pH of the CSF samples was adjusted to 7.6 using 1N HCl, 30 µl of sample was added into a final mix (50 µl) containing 0.5 mM 5,5'-Dithiobis (2-nitrobenzoic acid), 5 U/ml glutathione reductase, and 125 µM NADPH in 50 mM phosphate buffer with 5 mM EDTA (pH 7.4). Absorbance at 412 nm was recorded for 5 min, and GSSG was used as a standard. The assay was carried out in triplicate.

GGT Activity Assay (Pang et al. 2013)

The CSF samples were centrifuged in the concentration tubes until the volume decreased to 1/7th the starting volume, 8 µl concentrated CSF sample was added to 36 µl reagent solution containing five volumes of L-gamma-Glutamyl-p-nitroanilide (GPNA, 6.8 mM, pH 8.2) and one volume of Gly-Gly (344.5 mM, pH 8.2). The absorbance at 410 nm was measured for 1 h at 37 °C. One unit of activity was defined as the consumption of 1 µmol of GPNA per minute. Each sample was measured in triplicate.

GPx Activity Assay (Pang et al. 2013)

The concentrated CSF samples (6 µL) were added to a mixture (18 µL) containing 5 mM GSH, 5 µg/ml GR, and 0.26 mM NADPH in 50 mM potassium phosphate buffer with 5 mM EDTA (pH 7.4), followed by incubation at 37 °C for 3 min. The reaction was initiated by adding 2 µL of 6 mM tert-butylhydroperoxide. The absorbance at 340 nm was recorded for 10 min at 37 °C. One unit of enzyme activity was defined as the consumption of 1 µmol of NADPH per minute. Each sample was measured in triplicate.

4-Hydroxynonenal (HNE) Assay

The concentration of histidine-conjugated HNE was measured using OxiSelect™ HNE-His Adduct ELISA kits from Cell Biolabs (San Diego, CA).

Thiobarbituric Acid Reactive Substances (TBARS) Assay

CSF (10 µL) diluted with 40 µL PBS was mixed with 5 µL 2-Thiobarbituric acid (TBA) solution (67 mg/ml, in DMSO), incubated at 100 °C for 10 min, cooled to room temperature, and the absorbance was recorded at 532 nm. Malondialdehyde (MDA) was used as a standard.

Statistical Analysis

Software used included SAS Enterprise 4.3 (SAS Institute Inc, Cary, NC, USA) and Prism 5 (GraphPad Software Inc., La Jolla, CA). Data found to be non-normally distributed were log or square root transformed prior to further analyses. Two-way ANCOVA followed by Bonferroni post hoc test were performed, with HIV and Meth status as independent variables and age as a co-variable, for each of the oxidative stress markers. Relationships between continuous variables were examined using Pearson (for normally distributed data) or Spearman (for non-normally distributed data) correlations, and the significance of difference between the slopes was evaluated by ANCOVA.

Results

Participant Characteristics (Table 1)

The age and gender distributions were similar among the 4 groups. However, the Control group had the highest (98 %) while the HIV+Meth group had the lowest (70 %) percentage of Non-Hispanic participants, and more (>50 %) of the Control and HIV groups were White, compared to ~20 % in the Meth and HIV+Meth groups. The Meth group had the

Table 1 Demographic and clinical characteristics of the study subjects (mean±S.E.)

Group/variable	Control (n=41)	Meth (n=25)	HIV (n=34)	HIV+Meth (n=23)	p value ANOVA, T and X ²
Age (years)	39.7±2.0	39.1±2.0	41.8±1.9	43.0±1.6	0.54
Sex (Male/Female)	37 (90%) /4 (10%)	22 (88%) /3 (12%)	32 (94%) /2 (6%)	22 (96%) /1 (4%)	0.73
Ethnicity (Hispanic/Non-Hispanic)	1 (2%) /40 (98%)	4 (16%) /21 (84%)	5 (15%) /29 (85%)	7 (30%) /16 (70%)	0.019
Race:					
American Indian/Native Alaskan	1 (2%)	0 (0%)	1 (3%)	0 (0%)	0.032
Asian	8 (20%)	6 (24%)	4 (12%)	7 (30%)	
African American/Black	1 (2%)	0 (0%)	2 (6%)	3 (13%)	
Native Hawaiian/Pacific Islander	3 (7%)	5 (20%)	0 (0%)	2 (9%)	
White	22 (54%)	5 (20%)	18 (53%)	5 (22%)	
Mixed	6 (15%)	9 (36%)	9 (26%)	6 (26%)	
Clinical variables					
HIV Duration (months)			199.1±16.8	177.1±18.9	0.40
CD4 (cells/mL)			433.1±38.1	367.0±46.7	0.28
Nadir CD4 (cells/mL)			212.9±29.0	109.4±23.8	0.015
HIV Viral Load (VL) (log cp/mL)			2.8±0.3	2.6±0.3	0.64
Subjects with detectable VL			12 (35%)	10 (43%)	0.36
HIV Dementia Scale (0-16)			14.4±0.3	13.4±0.7	0.15
Karnofsky Scale (0-100)			92.1±1.5	90.9±1.4	0.59
CSF WBCs (cells/mm ³)	4.6±2.4	13.3±10.4	1.8±0.6	31.5±25.6	0.24
CSF Glucose (mg/dL)	60.9±1.0	60.9±1.2	61.2±3.3	62.0±1.9	0.99
HIV Medication (yes/no)			28 (82%) /6 (18%)	21 (91%) /2 (9%)	0.57
Methamphetamine usage					
Daily average Meth use (g)		0.91±0.04		0.98±0.03	0.78
Total lifetime Meth used (g)		3,771.3±174.9		3,464.4±184.1	0.81
Duration of Meth use (month)		206.6±4.3		195.1±5.4	0.73
Meth abstinence (month)		3.5±0.1		2.3±0.1	0.12
Other Drugs used					
Tobacco Smoking (yes/no)	30 (73%) /11 (27%)	19 (76%) /6 (24%)	24 (71%) /10 (29%)	12 (52%) /11 (48%)	0.26
Alcohol (yes/no)	37 (90%) /4 (10%)	23 (92%) /2 (8%)	28 (82%) /6 (18%)	18 (78%) /5 (22%)	0.41
Current Marijuana (yes/no)	10 (24%) /31 (76%)	4 (16%) /21 (84%)	10 (29%) /24 (71%)	6 (26%) /17 (74%)	0.69

Bold numbers indicate statistical significance

highest percentage of Native Hawaiian/Pacific Islanders (20 % vs. ≤9 % in other groups) while the HIV+Meth group had the highest percentage of African American/ Black (13 % vs. ≤6 % in other groups), and the HIV group had the lowest percentage of Asian (12 % vs. ≥20 % in other groups). The HIV+Meth group had lower nadir CD4 cell counts than the HIV group, which is consistent with prior reports of HIV+ stimulant users (Ellis et al. 2003; Shoptaw et al. 2012). Current usage of tobacco, alcohol and marijuana may also induce oxidative stress, but were found to be similar among the 4 groups.

CES-D and Its Correlations with HNE, Meth Use, HIV Dementia Scale, and Karnofsky Scale

Figure 2a shows that both HIV infection ($p<0.0001$, two-way ANCOVA) and Meth use ($p=0.01$) were associated with

higher CES-D score, and there was an interactive (not additive) effect between these two factors (interactive- $p=0.0015$).

HIV subjects had higher levels of HNE than controls in the CSF (Fig. 2b, $p_{\text{HIV}}<0.0001$, two-way ANCOVA). Untransformed data show that the HNE level in the HIV group was 3.5 folds higher than the Control group ($p<0.0001$, Bonferroni post hoc), and 3 folds higher than the Meth group ($p<0.0001$). Similarly, the HNE level in the HIV+Meth group was 2.25 folds higher than the Control group ($p<0.0001$), and 1.9 folds higher than the Meth group ($p=0.0004$). The HNE levels in the Control group and the Meth group were similar.

A strong positive correlation between CES-D and HNE was found in subjects who did not have a recent history of Meth use disorders (Control and HIV groups, $p=0.0003$, Fig. 2c), but the correlation was not found in the recently abstinent Meth users (Meth and HIV+Meth groups,

Fig. 2 CES-D score, HNE concentration in the CSF, and their correlations with each other and with Karnofsky scale and Meth use. **a** Square root-transformed CES-D score of subjects in the Control ($n=41$), HIV ($n=34$), Meth ($n=25$) and HIV+Meth ($n=23$) groups. **b** Log transformed HNE concentration in the CSF of the 4 groups. **c** Correlations between CES-D and HNE in Meth- (i.e. Control and HIV) and Meth+ (i.e., Meth and HIV+Meth) subjects. **d** Correlation of CES-D vs. Karnofsky scale in HIV-positive subjects (i.e. HIV and HIV+Meth). **e** Correlation of CES-D vs. duration of Meth use in recent Meth users (i.e., Meth and HIV+Meth). **f** Correlation of Log HIV dementia scale vs. Log HNE. The p values were calculated from Pearson correlations. In panels **a** and **b**, the p values were calculated from two-way ANCOVA. $**p<0.01$; $***p<0.0001$, Bonferroni post hoc test. In panels **c–f**, the p -values were calculated from Pearson or Spearman correlations

interaction- $p=0.0014$). The TBARS was measured in 29 of the CSF samples ($n=8$ for Control, $n=11$ for HIV, $n=7$ for Meth, and $n=3$ for HIV+Meth), as another assay of oxidative stress to corroborate the HNE findings; TBARS also correlated with CES-D across all subjects ($p=0.046$, $r=0.37$).

In the HIV-seropositive subjects, those with lower Karnofsky scale had more severe depressive symptoms (higher CES-D, $p=0.0346$, Fig. 2d). For the recently abstinent Meth users, those who had used Meth for a longer duration had less depressive symptom ($p=0.0285$, Fig. 2e). In the HIV+Meth group, but not the HIV group, more severe HIV dementia was associated with higher levels of oxidative stress, as assessed by HNE ($p=0.0017$, Fig. 2f). Conversely, in the HIV group, but not the HIV+Meth group, those with higher Karnofsky scale scores showed a trend for higher HIV dementia scores ($p=0.05$, $\text{Rho}=0.343$, Spearman's correlation), and a trend for less oxidative stress, as assessed by HNE ($p=0.03$, $\text{Rho}=-0.383$, Spearman's correlation).

Correlations Between GSH, GPx, HNE, and Meth Use

HIV-infected subjects had lower GSH concentrations in their CSF ($p_{\text{HIV}}=0.002$, two-way ANCOVA, Fig. 3a). Untransformed data show that GSH level in the HIV group was 34 % lower than the Control group ($p=0.04$), and 49 % lower than the Meth group ($p=0.004$), while the GSH level in the HIV+Meth group was 40 % lower than the Meth group ($p=0.01$). The Meth group had a non-significantly higher GSH level than the Control group (+30 %, $p=0.16$).

GSH synthesis is limited by the activity of gamma-glutamyl cysteine synthetase (GCS), and the gene expression (Iles and Liu 2005) and activity (Backos et al. 2011) of GCS can be upregulated by HNE. Positive correlations between HNE and GSH were found in HIV-negative subjects ($r=0.39$, $p=0.0015$), but not in those with HIV infection (interaction- $p=0.001$, Fig. 3b).

Fig. 3 GSH concentration and GPx activity in the CSF and their correlations with HNE and Meth use. **a** Log transformed GSH concentration in the CSF of subjects in the Control ($n=41$), HIV ($n=34$), Meth ($n=25$) and HIV+Meth ($n=23$) groups. **b** Different correlations between GSH and HNE in HIV+ (i.e., HIV and HIV+Meth) and HIV- (i.e., Control and Meth) subjects. **c–e** Correlations between GSH and total Meth use, daily meth use, and duration of Meth abstinence in recent Meth users (i.e., Meth and HIV+Meth). **f** GPx activity in the CSF of the 4 groups. **g** Different correlations between GPx and HNE in the HIV+ (i.e., HIV and HIV+Meth) and HIV- (i.e., Control and Meth) subjects. In panels **a** and **f**, the p value was calculated from two-way ANCOVA. * $p<0.05$, ** $p<0.01$, Bonferroni post hoc test. In the other panels, the p values were calculated from Pearson correlations

For the recently abstinent Meth users, those who used greater lifetime cumulative amounts of Meth ($p=0.0003$, Fig. 3c), used greater amounts of Meth daily ($p=0.0073$,

Fig. 3d), or had been recently abstinent for longer period ($p=0.025$, Fig. 3e), showed higher GSH concentration in their CSF.

Recently abstinent Meth users had higher GPx activity in the CSF ($p_{\text{Meth}}=0.001$, two-way ANCOVA, Fig. 3f). Compared to the Control group, GPx activities were 23 % higher in both the Meth group ($p=0.009$) and HIV+Meth group ($p=0.04$), but similar in the HIV group.

Since GPx reduces lipid peroxidation, HNE generation may be decreased by GPx. Amongst the HIV-positive subjects, those with higher GPx indeed showed lower HNE ($p=0.002$, Fig. 3g), but the HIV-negative groups with relatively lower HNE levels showed no such correlations (interaction- $p=0.008$).

GGT Activity and Its Correlations with GSH, GPx, CES-D, and Age

HIV-positive subjects had higher CSF GGT activity than HIV-negative subjects ($p_{\text{HIV}}=0.0018$, two-way ANCOVA, Fig. 4a). Untransformed data show that compared to the Control group, the GGT activity was 27 % higher in the HIV group ($p=0.02$) and 37 % higher in the HIV+Meth group ($p=0.01$). The HIV+Meth group also showed a 32 % higher GGT activity than the Meth group ($p=0.01$).

Since GSH catabolism in the CSF can be catalyzed by GGT, we further correlated GSH concentration to GGT activity, and greater GGT activities indeed correlated with lower GSH levels across all subjects ($r=-0.19$, $p=0.05$, Fig. 4b),

especially in the recently abstinent Meth users who had the highest GSH.

Across the 4 groups, those with higher GGT activity also had higher GPx activity ($p<0.0001$, Fig. 4c), and were older ($p<0.0001$, Fig. 4d).

Discussion

Depression and Oxidative Stress

Higher levels of oxidative stress markers were observed in the plasma of depressed patients (Ozcan et al. 2004; Dimopoulos et al. 2008; Maes et al. 2010); however, whether these markers correlate with the severity of depression remains controversial (Dimopoulos et al. 2008; Maes et al. 2010). In our study, the CES-D scores of subjects who did not have recent Meth use, regardless of their HIV status, showed strong correlation with lipid peroxidation (HNE levels) in the CSF. Similar to other studies that showed increased lipid peroxidation in the CSF and brain tissues of HIV patients (Castagna et al. 1995; Sacktor et al. 2004), we also found increased HNE in the CSF of HIV-positive subjects. Therefore the high prevalence of depressive symptoms in people living with HIV is at least partially related to increased oxidative stress in the CNS.

Fig. 4 GGT activity in the CSF and its correlations with GSH, GPx, and age. **a** GPx activity in the CSF of subjects in the Control ($n=41$), HIV ($n=34$), Meth ($n=25$) and HIV+Meth ($n=23$) groups. **b** Correlations between GGT and GSH. **c** Correlations between GGT and GPx. **d** Correlations between GGT and age. In panel **a**, the p value was calculated from two-way ANCOVA. *** $p<0.001$, Bonferroni multiple comparison test. In panels **b–d**, the p values were calculated from Pearson correlations. *n.s.* non-significant

Depressive Symptoms and Subjects with Meth Use Disorders

Although recent Meth users had greater depressive symptoms on CES-D scores than the controls, the CES-D scores did not correlate with HNE. These findings suggest that depressive symptoms in the Meth users is likely mediated through other mechanisms (e.g., dysregulated neurotransmitters and neuro-inflammation), and less related to oxidative stress in the CNS. The Meth users with longer duration of Meth use had less depressive symptoms, with lower CES-D scores, which suggest a potential adaptation to Meth exposure. In our study, the HIV+Meth group had similar CES-D scores as the HIV group, which differs from a previous report of more depressive symptoms in the HIV+Meth group than HIV, Meth or controls, when assessed by the Beck Depression Inventory-I (Bousman et al. 2009). The discrepant findings between the 2 studies might be due to differences in the subject sample and characteristics, such as younger age, lower education or socioeconomic status, and shorter total duration of Meth use or active Meth use in the HIV+Meth subjects in the prior study.

Meth Use and Oxidative Stress

Preclinical studies typically reported Meth-induced increases of HNE in the brain (Flora et al. 2002; Cadet et al. 2009; Koriem et al. 2013). However, in our study, the recent Meth users had relatively normal CSF HNE levels, while the HIV+Meth group even had lesser elevation of HNE than the HIV group. Therefore, the Meth-mediated compensatory antioxidant response (such as increases in GPx and GSH) might have contributed to the amelioration of oxidative stress in the CSF, possibly due to the chronic (~17 years) regular Meth use by these subjects, which is different from acute Meth administration in the animal studies.

GSH in Relation to HIV Infection and Meth Use

Lower serum GSH levels were detected in schizophrenic patients (Looney and Childs 1934), and in depressed women (Kodydkova et al. 2009). The GSH depletion in the CSF of HIV-positive subjects in our study is consistent with prior reports that found decreased GSH levels in the CSF and brain tissues of HIV patients (Sacktor et al. 2004; Bandaru et al. 2007). In the HIV-positive subjects, the lack of correlation between GSH and HNE suggests that the chronically HIV-infected CNS might not be able to upregulate the GSH level in response to increased oxidative stress. Although the molecular pathway for how HIV affects GSH metabolism in the CNS is not known, HIV viral proteins modulated GCS (the rate-limiting enzyme for GSH metabolism) expression and activity in mouse muscle and liver (Choi et al. 2000).

Some animal studies showed temporary increases of GSH in the brain after acute Meth exposure (Harold et al. 2000;

Flora et al. 2002). In our study, the CSF GSH level was 30 % higher in the recent Meth users compared to Controls, and the GSH levels were elevated in proportion to the daily and cumulative amounts of Meth used. These findings suggest that long-term chronic Meth use resulted in compensatory increases of GSH in response to the ongoing oxidative stress. Interestingly, the Meth users with higher CSF GSH levels also had longer duration of Meth abstinence (average 3 months); hence, this compensatory response may persist for many months even after cessation of Meth use.

GPx in Relation to HIV and Meth

Depressed patients showed lower serum GPx activity (Kodydkova et al. 2009), especially in those with greater severity of depression (Maes et al. 2011). However, no correlation was found between CES-D scores and CSF GPx activity in our subjects. The discrepancy between our study and others' may be related to the different GPx isoforms in the blood (cytosolic GPx1, and membrane- and mitochondrion-located GPx4) versus those in the CSF (extracellular GPx3).

The negative correlation between GPx activity and HNE in the CSF of HIV-positive subjects suggests a potential role of GPx in preventing HNE formation during chronic HIV infection. However, this protective effect may be limited by the low GPx content in the CSF (<1 % of that in the serum) (Solovyev et al. 2013), and depletion of CSF GSH during HIV infection. Our HIV subjects had relatively normal GPx activity in the CSF, probably due to their relatively well-controlled viral load and very mild brain injury, as evidenced by their minimal cognitive deficits with relatively high scores on the HIV dementia scale. This result is consistent with a previous study that found normal GPx activity in the CSF of asymptomatic HIV patients (Velazquez et al. 2009).

Several studies documented that Meth affected GPx activities in rodent brain tissues, and the changes were dependent on the dosage and frequency of Meth exposure, and the brain region studied (Flora et al. 2002; Cadet et al. 2009; Koriem et al. 2013). Our study showed that chronic Meth abuse was associated with upregulated GPx activity in the CSF, and this increase along with elevated GSH concentration may effectively counteract against the oxidative stress caused by Meth.

GGT in Relation to GSH, GPx, and Age

The positive correlation between GGT and GPx in the CSF, across all subjects, has not been documented before. Our HIV subjects showed higher GGT activity in the CSF, which parallels the higher serum GGT activities reported in HIV patients (VayÃ et al. 2012). We also found that higher GGT activity was associated with GSH depletion in the CSF, which likely resulted from GGT-mediated extracellular GSH decomposition. Both *GGT* and *GPX* genes contain antioxidant response

elements with binding sites for Nrf2 (Zhang et al. 2006; Westphal et al. 2009), which can be activated by oxidative stress (Kensler et al. 2007), and is required for basal *GPX3* expression, and for *GPX3* and *GGT* upregulation (Zhang et al. 2006; Westphal et al. 2009). Therefore, future studies of oxidative stress in HIV subjects and Meth users should evaluate whether Nrf2 activation leads to simultaneous upregulation of GPx and GGT in the CSF.

The age-dependent increase in CSF GGT found in our study also has not been reported previously. The relationship between GGT and age is tissue-specific. For example, GGT activity was increased in several brain regions in aged rats (Zhu et al. 2007), and in sera of aged humans (Hsu et al. 1996); but decreased in muscle (Chen et al. 2010), kidney and lung (Jenkinson et al. 1991) in aged rats. Since Nrf2 activation is involved in upregulation of *GGT*, and was shown to increase with age in mouse brains (Zhang et al. 2012), it may also have a regulatory role in the age-related changes of GGT.

Relationships Between Meth Use and HIV on General Well Being and Cognitive Screen

HIV subjects with recent Meth use maintained their Karnofsky scores (general well being) at a constant high level (~90) regardless of their HIV dementia scale or oxidative stress in the CNS, which is different from HIV subjects without recent Meth use, whose well being declined with worse cognitive function (on the HIV dementia scale) and higher levels of oxidative stress. The high level of Karnofsky score in the HIV+Meth group may be due to a bias subject selection based on their recruitment source. However, this finding also suggests that Meth may be used as “self-medication” to maintain their general well being, which may contribute to the high prevalence of Meth use in the HIV-infected population (Semple et al. 2002). On the other hand, poorer cognition (on the HIV dementia scale) was associated with greater oxidative stress (higher CSF HNE) only in the HIV+Meth group, but not the HIV group, which may be related to the greater neuroinflammation often found in HIV+Meth subjects than in HIV subjects (Chang et al. 2005), and the greater oxidative stress also may contribute to greater neuroinflammation (Meulendyke et al. 2014).

Limitations of this Study

The limitations of this study include: (1) the cross-sectional design provides only associations but not direct causal links between HIV, with or without Meth use, and the altered levels of CSF oxidative stress markers. (2) The GSH and oxidative stress markers in the CSF samples only reflect the extracellular environment of the brain, which may be different from those in the cells. Therefore, direct measurements

of oxidative stress levels in the brain tissue would provide a stronger association between the severity of depressive symptoms and the levels of oxidative stress in the CNS.

In conclusion, both HIV infection and Meth use disorders were associated with greater depressive symptoms, an interactive effect between these two conditions was found on depressive symptoms, but no interactive or additive effects were found on oxidative stress. The differential relationship between depressive symptoms and oxidative stress response amongst the four subject groups suggest that depressive symptoms in these groups are mediated through different mechanisms which are not always related to oxidative stress. Greater depressive symptoms were associated with higher levels of oxidative stress in the CSF of HIV patients, while individuals with recent Meth use had higher levels of GPx and GSH in their CSF, and a greater capacity to compensate for the oxidative stress.

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Conflict of Interest The authors declare that there are no conflicts of interest.

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