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3,4-Methylenedioxymethamphetamine (MDMA) Abuse may Cause Oxidative Stress and Potential Free Radical Damage

JUN F. ZHOU[a,b,*], PENG CHEN[a], YE H. ZHOU[c], LIANG ZHANG[c] and HUAI H. CHEN[a,b]

[a]*Second Affiliated Hospital, College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou, 310009, Zhejiang Province, People's Republic of China;* [b]*Zhejiang Surveillance Station of Drug Abuse, Hangzhou, Zhejiang Province, People's Republic of China;* [c]*Hangzhou Municipal Public Security Bureau, 35 Huaguang Road, Hangzhou, 310002, Zhejiang Province, People's Republic of China*

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Objective: To investigate whether 3,4-methylenedioxymethamphetamine abuse (MDMA abuse) may cause oxidative stress and potential free radical damage in the bodies of MDMA abusers (MA), and to explore the mechanisms by which MDMA abuse may be causing oxidative stress.

Methods: One hundred and twenty MA and 120 healthy volunteers (HV) were enrolled in a random control study design, in which the level of lipoperoxide (LPO) in erythrocytes, and the levels of Vitamin C (VC), Vitamin E (VE) and β-carotene (β-CAR) in plasma as well as the activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes were determined by spectrophotometric methods.

Results: Compared with the average values of the above biochemical parameters in the HV group, the average value of LPO in erythrocytes in the MA group was significantly increased ($P < 0.0001$), while the average values of VC, VE and β-CAR in plasma as well as those of SOD and CAT in erythrocytes in the MA group were significantly decreased ($P < 0.0001$). The analysis of bivariate correlations suggested that with the increase of the MDMA abuse dose and the MDMA abuse duration, the level of LPO in erythrocytes in the MA was increased ($P < 0.0001$), while the levels of VC, VE and β-CAR in plasma as well as the activities of SOD and CAT in erythrocytes in the MA were decreased ($P < 0.0001$).

Conclusion: The findings in this study suggest that MDMA abuse may cause oxidative stress and potential free radical damage to MA.

Keywords: 3,4-Methylenedioxymethamphetamine; MDMA abuse; Oxidative stress; Free radicals; Oxidation; Lipoperoxidation

INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA) is a substituted amphetamine with stimulating and hallucinogenic properties. Since MDMA is "ecstasy", it is used extensively as a "recreational" drug and is ingested by young people. It is well established that MDMA is neurotoxic and produces long term degeneration of cerebral 5-hydroxytryptamine (5-HT) nerve terminals in many species, diminution of antioxidant capacity in the brain, decrease of antioxidants such as Vitamin C (VC) and Vitamin E (VE), aggravation of oxidative stress, and occurrence of oxidative damage and lipoperoxidative damage resulting from excessive free radical formation and abnormal free radical reactions in many animal experiments.[1–25] However, up to now, there have been neither reports on abnormal free radical chain reactions in humans abusing MDMA, nor reports about any relationship between oxidative stress, free radical damage and MDMA abuse. To investigate whether MDMA abuse may cause oxidative stress and potential free radical damage in the bodies of MDMA abusers (MA), and to explore the mechanisms by which MDMA abuse may be causing oxidative stress, 120 MA and 120 healthy volunteers (HV) were enrolled in a random control study design, in which the level of lipoperoxide (LPO) in erythrocytes, and the levels of VC, VE and β-carotene

*Corresponding author. Tel.: +86-571-87783768. Fax: +86-571-87213864. E-mail: hzzjf@hotmail.com

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(β-CAR) in plasma as well as the activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes were determined by spectrophotometric methods. At the same time, the differences between the average values of the biochemical parameters in the MA group and the HV group are compared, and the bivariate correlations between the MDMA abuse dose and the biochemical parameters as well as the MDMA abuse duration and the biochemical parameters are analyzed.

MATERIALS AND METHODS

Study Design

A random control study design was used in the present study. In order to obtain an objective research conclusion, the principles of random, control, replication and equilibrium, and the management factor, experimental effect and subjects, and the inclusion criteria and exclusion criteria of subjects, etc. were taken into full consideration, and were strictly executed in the studying practice.[26]

Subjects

MDMA Abusers (MA)

\One hundred and twenty MA were randomly sampled from 217 MA who continuously abused MDMA for more than 1 month, and were diagnosed and confirmed by means of a Chemtrue™ urine-MDMA one-step test, with self-confession. The MDMA doses they abused daily were 40 – 120 mg (80.75 ± 19.96). Analyzed by HPLC the MDMA content per tablet of ecstasy ranged from 8.75 to 47.53 mg, and MDMA abuse duration was 1 – 12 months (5.8 ± 2.9). They were all volunteers in this study.

Healthy Volunteers (HV)

One hundred and twenty HV were randomly sampled from 250 HV confirmed by comprehensive physical examination at the Second Affiliated Hospital, College of Medicine, Zhejiang University, with "Select Cases—Random Sample" of "SPSS 11.0 for Windows". They were all volunteers in this study. They had not abused any drugs, sedative-hypnotics and others, and their Chemtrue urine tests were negative.

The demographic data and some other data of 120 MA and 120 HV are presented in Table I.

The above MA and HV' medical history of disorders associated with brain, heart, lung, liver, kidney and other organs as well as blood system, circulatory system, respiratory system, digestive system and other systems were all excluded by their routine blood, urine and feces examinations as well as radiographs, cardiogram and other necessary examinations. History of inflammation, hypertension, hyperlipidemia, acute or chronic bronchitis, autoimmune disease, diabetes, atherosclerosis, tumors and other diseases, and subnutrition, malnutrition, supernutrition and other nutritional diseases were also all excluded. In addition, the subjects had no smoking or excessive drinking history.

The above subjects were never exposed to radiation, nor engaged in work exposing them to intoxicating materials or pesticides. Within the prior month in which they volunteered the experimentation in the study, none of the subjects had taken any antioxidant supplements such as VC, VE, β-CAR,

TABLE I The demographic data and some other data in the MA group and the HV group

Item	MA ($n = 120$)	HV ($n = 120$)	Statistic analysis
Age (year)	18–35	20–35	$t = 0.179$*
	(23.5 ± 3.4)	(23.6 ± 3.0)	$P = 0.858$
Gender	M = 67	M = 60	$\chi^2 = 0.819$**
	F = 53	F = 60	$P = 0.365$
Systolic pressure (mmHg)	88–132	85–134	$t = 0.511$*
	(110.82 ± 11.19)	(110.10 ± 10.52)	$P = 0.610$
Diastolic pressure (mmHg)	60–86	62–84	$t = 0.342$*
	(75.57 ± 6.73)	(75.29 ± 6.15)	$P = 0.733$
Hemoglobin (g/l)	115–148	120–153	$t = 0.782$*
	(134.94 ± 6.96)	(135.66 ± 7.19)	$P = 0.435$
Albumin (g/l)	35.21–50.22	35.84–51.38	$t = 0.762$*
	(42.62 ± 2.30)	(42.85 ± 2.40)	$P = 0.447$
Body-mass index	19.34–25.36	19.49–25.51	$t = 0.450$*
	(23.18 ± 1.26)	(23.25 ± 1.33)	$P = 0.653$
Smoking history	No	No	–
Abusing alcohol history	No	No	–

Note: *Independent samples *t* test, **Pearson chi-square test.

ginkgo biloba, tea polyphenols of other similar substances.

METHODS

Collection and Pretreatment of the Blood Samples

Fasting venous blood samples from elbow in left arm were collected in the morning from all the subjects and heparin sodium was added as anticoagulant, and the promptly separated plasma and erythrocytes were stored at −50°C immediately.[27–29] The blood samples collected did not undergo any hemolysis.

Biochemical Measurements

Erythrocyte LPO Level

Spectrophotometry of thiobarbituric acid reactive substances (TBARS) was used to determine erythrocyte LPO level which was expressed as nmol/g Hb.[27,28]

Plasma VC Level

Trichloroacetic acid solution was used to sediment proteins in plasma and to extract VC from plasma. The VC in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine to form a colored end product that was detected at 563 nm, with its level expressed as μmol/l.[27,28]

Plasma VE Level

Absolute ethanol was used to sediment proteins in plasma and to extract VE from plasma. The VE in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine to form a colored end product that was detected at 563 nm, with its level expressed as μmol/l.[27–29]

Plasma β-CAR Level

β-CAR was extracted with a mixture of ethanol and petroleum ether, and was assayed with spectrophotometry, and its level was expressed as μmol/l.[27,28]

Erythrocyte SOD Activity

Spectrophotometry of inhibiting pyrogallol autooxidation was used to determine erythrocyte SOD activity which was expressed as U/g Hb.[27,28]

Erythrocyte CAT Activity

Spectrophotometry of coloration of hydrogen peroxide and acetic acid–potassium dichromate was used to determine erythrocyte CAT activity which was expressed as K/g Hb.[27,28]

In the determination of the above biochemical substances and enzymes, the main analytical reagents, such as VC, VE, β-CAR, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonic acid disodium salt (ferrozine), Cu/Zn-SOD, CAT, 1,2,3-trihydroxybenzene (pyrogallol), 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid (TBA), were purchased from SIGMA® chemical company, USA; and the other analytical reagents were produced in China, the fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. In the determination of the above biochemical substances and enzymes, the main analytical instruments included Hewlett Packard 8453-Spectrophotometer, USA, and others.

The kit used as diagnosis and confirmation of every MA was Chemtrue™ urine-MDMA one-step test kit for *in-vitro* diagnosis use, and its sensitivity was 500 ng/ml, made in Medyl Biotechnology, Inc., San Diego, CA, USA. There were no significant differences in the sensitivity and precision between the Chemtrue™ urine-MDMA one-step test and the HPLC, and no significant differences in the false positives and false negatives between the HPLC and the Chemtrue™ urine-MDMA one-step test, according to our experiment.

In the determination of the above biochemical substances and enzymes, the standardization of experiment, e.g. the same batch number of each reagent, the same quality control, the same lab assistant, and the identical analytical apparatus were strictly used for every experiment in order to decrease errors, and to ensure the analytical quality of determinations.[26–29]

Medical Statistical Analysis

All data were statistically analyzed with SPSS 11.0 for Windows static software using a Compaq Pentium IV/1.6 GHz computer. The biochemical parameters in this study presented normal distributions by Kolmogorov–Smirnov Z test, and were expressed as mean ± standard deviation ($\bar{\chi} \pm s$) and 95% confidence interval (95% CI). Hypothesis testing methods included independent-samples t test, Pearson chi-square test (χ^2 test), and Pearson product-moment correlation analysis. In the statistical analysis of this study, the level of hypothesis testing (α) was ≤ 0.05 in order to avoid false positives (Type I error), and the power of hypothesis testing (power) was ≥ 0.80 to avoid false negatives (Type II error).[26]

TABLE II Comparison between the average values ($\bar{\chi} \pm s$) of the biochemical parameters in the MA group and in the HV group

		Oxidative constituents	Antioxidative constituents				
		Erythrocytes	Plasma			Erythrocytes	
Group	*n*	LPO (nmol/g Hb)	VC (μmol/l)	VE (μmol/l)	β-CAR (μmol/l)	SOD (U/g Hb)	CAT (K/g Hb)
MA	120	39.31 ± 4.54 (38.48–40.13)	36.41 ± 9.67 (34.66–38.15)	15.90 ± 4.15 (15.15–16.65)	1.19 ± 0.32 (1.13–1.25)	1838 ± 146 (1812–1865)	248.6 ± 65.2 (236.8–260.4)
HV	120	27.96 ± 3.90 (27.25–28.66)	55.43 ± 14.00 (52.90–57.96)	25.78 ± 6.16 (24.67–26.89)	1.72 ± 0.46 (1.64–1.80)	2126 ± 165 (2096–2156)	309.2 ± 81.4 (294.5–323.9)
*t**		20.771	12.247	14.573	10.381	14.319	6.363
P		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: *Independent-samples *t* test. The figures in parentheses are 95% confidence interval.

RESULTS

Comparison Between the Average Values ($\bar{\chi} \pm s$) of the Above Biochemical Parameters in the MA Group and the HV Group, and 95% CI

Compared with the average values of the biochemical parameters in the HV group, the average value of LPO in erythrocytes in the MA group was significantly increased, while the average values of VC, VE and β-CAR in plasma as well as those of SOD and CAT in erythrocytes in the MA group were significantly decreased (Table II). The lower limit of 95% CI of the average value of LPO in the MA group was greater than the upper limit of 95% CI of the average value of LPO in the HV group, and the upper limits of 95% CI of the average values of VC, VE, β-CAR, SOD and CAT in the MA group were less than the lower limits of 95% CI of those in the HV group (Table II).

Analysis of Bivariate Correlations Between the MDMA Abuse Dose and the Every Biochemical Parameter for 120 MA

The Pearson product-moment correlation analysis suggested that with the increase of MDMA abuse dose the level of LPO was increased gradually, while the levels of VC, VE, β-CAR, SOD and CAT were decreased gradually (Table III).

Analysis of Bivariate Correlations Between the MDMA Abuse Duration and the Every Biochemical Parameter for 120 MA

The Pearson product-moment correlation analysis suggested that with the increase of MDMA abuse duration the level of LPO was increased gradually, while the levels of VC, VE, β-CAR, SOD and CAT were decreased gradually (Table IV).

DISCUSSION

The findings in the present study suggested that MDMA abuse might cause oxidative stress and potential free radical damage to MA. There might be several interpretations.

MDMA abuse elicits hyperthermia, a potentially deleterious condition that aggravates its direct toxic effects.[6,7,11–13,15,16] Hyperthermia could be an important reason why a series of free radicals (FRs), such as $O_2^{\bullet -}$, $^{\bullet}OH$ and other FRs, is generated. For this reason and because of, the depletion of glutathione (GSH) induced by MDMA oxidative stress might be caused, thus producing the lipoperoxidation of polyunsaturated fatty acids (PUFAs) in the cellular membranes, increasing the content of LPO, decreasing GSH content, causing free radical damage of cells in the liver as well as the hippocampus, striatum and cortex in the brain.[6,7,11–13,15,16] At the same time, FRs can be

TABLE III Linear correlation analysis between the MDMA abuse dose and the every biochemical parameter

Correlative item	*n*	Correlative coefficient (*r*)	*t*	*P**
MDMA abuse dose with erythrocytic LPO	120	0.7330	11.7062	<0.0001
MDMA abuse dose with plasma VC	120	−0.5705	7.5459	<0.0001
MDMA abuse dose with plasma VE	120	−0.4147	4.9506	<0.0001
MDMA abuse dose with plasma β-CAR	120	−0.5528	7.2071	<0.0001
MDMA abuse dose with erythrocytic SOD	120	−0.5350	6.8791	<0.0001
MDMA abuse dose with erythrocytic CAT	120	−0.6362	8.9571	<0.0001

*Pearson product-moment correlation analysis.

TABLE IV Linear correlation analysis between the MDMA abuse duration and the every biochemical parameter

Correlative item	*n*	Correlative coefficient (*r*)	*t*	*P**
MDMA abuse duration with erythrocytic LPO	120	0.6393	9.0307	<0.0001
MDMA abuse duration with plasma VC	120	−0.4665	5.7296	<0.0001
MDMA abuse duration with plasma VE	120	−0.4597	5.6233	<0.0001
MDMA abuse duration with plasma β-CAR	120	−0.4444	5.3884	<0.0001
MDMA abuse duration with erythrocytic SOD	120	−0.4744	5.8538	<0.0001
MDMA abuse duration with erythrocytic CAT	120	−0.5683	7.5028	<0.0001

*Pearson product-moment correlation analysis.

formed during metabolism of MDMA and autoxidation of MDMA metabolism might explain why a large number of FRs were generated and oxidative stress occurred after MDMA was ingested by the abusers.[5–7,11–13,15,16] Additionally, the decrease of plasma VE level could lead to increased erythrocyte LPO level.[27–29]

MDMA abuse induces an acute release of 5-HT and dopamine (DA), followed by depletion of intraneuronal 5-HT stores, the initially released 5-HT stores, the initially released 5-HT activated postsynaptic 5-HT2A/2C receptors located on gamma-aminobutyric acid (GABA) interneurons, resulting in the decrease in GABAegic transmission and the increase of DA release and synthesis.[8,9,12–14,23,25] The excessively released DA can be transported into the depleted 5-HT terminal, at the same time, the excessive DA is deaminated by monoamine oxidase B (MAO-B) located within the 5-HT terminal, thereby resulting in the generation of a large number of FRs and reactive oxygen species (ROS), leading to lipoperoxidation and other oxidative stress in cellular membranes and potential free radical damage.[8,9,12–14,23,25] Therefore, such phenomena might occur when endogenous free radical scavenging mechanism became overwhelmed or exhausted.[13,27–45]

It should be pointed out that VC, VE, β-CAR, and so on, have to be acquired from dietary sources because they cannot be synthesized in the human body, whereas, antioxidative enzymes such as SOD, CAT, etc. are mainly synthesized in the human body, not from dietary sources.[27–33,35,37,38,43–45] Long and high dose MDMA abuse produces a series of digestive-systemic toxic reactions such as anorexia or loss of appetite, nausea, vomiting and diarrhea, which could decrease their intake, thereby accelerating free radical damage and the occurrence of oxidative stress. Long and high dose MDMA abuse also produces circulatory-systemic toxic symptoms such as palpitation, arrhythmia, and elevation or fall of blood pressure, and neurotoxic symptoms such as dysphoria, anxiety, tension, depression, tremor and vertigo, swoon, collapse, vague mind, mania, and so on. These might promote the generation and release of excessive FRs by means of xanthine–xanthine oxidase system, and by the effects of cytokines, especially interleukin-1, and by abnormal metabolism of cytochromes P-450, particularly cytochrome P-450 2E1.[27,30,35,37,43] However, body mass indexes were the same in abusers and controls (Table I).

The findings of bivariate correlation analysis between the MDMA abuse dose and the every biochemical parameter as well as those between the MDMA abuse duration and every biochemical parameter suggests that the MDMA abuse dose and duration are related to oxidative stress and potential free radical damage. Namely, the longer a person abused MDMA and the more a person abused MDMA daily, the bigger was the likelihood of oxidative stress and potential free radical damage.

Undoubtedly, limitations exist in the present study, e.g. the exact intake quantity and the exact quantity of MDMA in blood or in urine in the MA were not confirmed by HPLC. The present study is thus indirect evidence that MDMA abuse may induce oxidative stress and potential free radical damage.

In conclusion, the findings in the present study suggest that MDMA abuse may cause the aggravation of a series of free radical chain reactions and the possible oxidation stress in the bodies of MA, thereby resulting in the potential free radical damage in their bodies.

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