3,4-Methylenedioxymethamphetamine (MDMA) Abuse Markedly Inhibits Acetylcholinesterase Activity and Induces Severe Oxidative Damage and Liperoxidative Damage

JUN-FU ZHOU[*#1], YE-HUA ZHOU[‡], LIANG ZHANG[‡], HUAI-HONG CHEN[*#], AND DONG CAI[*]

[*]Second Affiliated Hospital, College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, Zhejiang Province, China; [#]Zhejiang Surveillance Station of Drug Abuse, 88 Jiefang Road, Hangzhou 310009, Zhejiang Province, China; [‡] Hangzhou Municipal Public Security Bureau, 35 Huaguang Road, Hangzhou 310002, Zhejiang Province, China

Objective To investigate whether 3,4-methylenedioxymethamphetamine (MDMA) abuse produces another neurotoxicity which may significantly inhibit the acetylcholinesterase activity and result in severe oxidative damage and liperoxidative damage to MDMA abusers. **Methods** 120 MDMA abusers (MA) and 120 healthy volunteers (HV) were enrolled in an independent sample control design, in which the levels of lipoperoxide (LPO) in plasma and erythrocytes as well as the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and acetylcholinesterase (AChE) in erythrocytes were determined by spectrophotometric methods. **Results** Compared with the average values of biochemical parameters in the HV group, those of LPO in plasma and erythrocytes in the MA group were significantly increased ($P<0.0001$), while those of SOD, CAT, GPX and AChE in erythrocytes in the MA group were significantly decreased ($P<0.0001$). The Pearson product-moment correlation analysis between the values of AChE and biochemical parameters in 120 MDMA abusers showed that significant linear negative correlation was present between the activity of AChE and the levels of LPO in plasma and erythrocytes ($P<0.0005$–0.0001), while significant linear positive correlation was observed between the activity of AchE and the activities of SOD, CAT and GPX ($P<0.0001$). The reliability analysis for the above biochemical parameters reflecting oxidative and lipoperoxidative damages in MDMA abusers suggested that the reliability coefficient (alpha) was 0.8124, and that the standardized item alpha was 0.9453. **Conclusion** The findings in the present study suggest that MDMA abuse can induce another neurotoxicity that significantly inhibits acetylcholinesterase activity and aggravates a series of free radical chain reactions and oxidative stress in the bodies of MDMA abusers, thereby resulting in severe neural, oxidative and lipoperoxidative damages in MDMA abusers.

Key words: 3,4-Methylenedioxymethamphetamine; MDMA; Drug abuse; Acetylcholinesterase; Free radicals; Lipoperoxide; Antioxidase; Oxidative stress; Oxidative damage; Lipoperoxidative damage

INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA) is a substituted amphetamine with

[1]Correspondence should be addressed to Prof. Jun-Fu ZHOU, Second Affiliated Hospital, College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou, 310009, Zhejiang Province, China. Tel: 86-571-87783768; Fax: 86-571-87213864; E-mail: zhuojun88@sina.com

Biographical note of the first author: Jun-Fu ZHOU, male, born in 1945, M.D., Research Scientist & Professor, majoring in preventive medicine, clinical medicine and free radical medicine.

0895-3988/2003
CN 11-2816

stimulating and hallucinogenic properties. Since MDMA induces "ecstasy", it is extensively used as a "recreational" drug and is ingested by young people. It has been well established that MDMA is neurotoxic and can result in long term degeneration of cerebral 5-hydroxytryptamine (5-HT) nerve terminals in many species, diminution of antioxidant capacity of the brain, decrease of anti-vitamins such as vitamin C and E, aggravation of oxidative stress, and occurrence of oxidative and lipoperoxidave damages due to excessive free radical formation and abnormal free radical reactions in many animal experiments[1-25]. However, up to now, there are neither reports on abnormal metabolism of MDMA-induced acetylcholinesterase activity and abnormal free radical chain reactions in the bodies of MDMA abusers, nor reports about relationship between oxidative stress, oxidative damage and MDMA abuse. To investigate whether MDMA abuse induces another neurotoxicity that significantly inhibits acetylcholinesterase activity and results in severe oxidative and liperoxidative damages in the human bodies, 120 MDMA abusers (MA) and 120 healthy volunteers (HV) were enrolled in an independent sample control design, in which the levels of lipoperoxides (LPO) in plasma and erythrocytes as well as the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and acetylcholinesterase (AChE) in erythrocytes were determined by spectrophotometric methods. In addition, differences between the average values of biochemical parameters in the MA group and the HV group were analyzed, the Pearson product-moment correlation between AChE activity and the above biochemical parameters for 120 MDMA abusers was determined, and the reliability analysis for the values of LPO in plasma and erythrocytes as well as for those of SOD, CAT and GPX in erythrocytes reflecting oxidative and lipoperoxidative damages of MDMA abusers was conducted.

MATERIALS AND METHODS

Subjects

MDMA abusers (MA). With "Select Cases-random Sample of Cases" in "SPSS 11.0 for Windows", 120 MDMA abusers (67 males and 53 females) were randomly selected from 217 MDMA abusers who, as confessed by themselves, used MDMA for more than 1 month in succession, were diagnosed and confirmed by Chemtrue™ urine-MDMA one-step test. Their ages ranged from 18 to 35 (23.5±3.4) years, the daily dosage of MDMA ranged from 40 to 120 (80.75±19.96) mg, and the duration of MDMA use ranged from 1 to 12 (5.8±2.9) months. The diseases associated with brain, heart, lung, liver, kidney and other organs were excluded, and so were the diseases associated with hypertension, hyperlipoidemia, chronic bronchitis, autoimmune disease, diabetes, atherosclerosis and tumors according to their medical history. They were all volunteers in this study.

Healthy volunteers (HV). By means of "Select Cases-Random Sample of Cases" in "SPSS 11.0 for Windows", 120 healthy volunteers (60 males and 60 females) were randomly selected from 250 healthy volunteers. Their ages ranged from 20 to 35 (23.6±3.0) years. They were found to be normal in their routine blood, urine and feces examinations and radiographs, and the disorders associated with brain, heart, lung, liver, kidney and other organs as well as hypertension, hyperlipoidemia, chronic bronchitis, autoimmune disease, diabetes, atherosclerosis and tumors were excluded. They were all volunteers in this study.

No significant difference was found between the average values for age in the MA group and the HV group by t test ($t = 0.179$, $P = 0.858$), and between the sex proportions in

the MA group and the HV group by c^2 test ($c^2 = 0.819$, $P = 0.365$).

Within the previous month, all the subjects had not taken any antioxidant supplements such as vitamin C, vitamin E, β-carotene, ginkgo biloba, theo-polyphenols or other similar substances before they were enrolled as volunteers in this study. They, in general, had not taken fruits or greenstuff containing rich anti-oxidative vitamins because they had a poor appetite due to continuous abuse of MDMA.

Methods

Collection and pretreatment of blood samples. Fasting venous blood samples were collected in the morning with heparin sodium added as anticoagulant, and the separated plasma and erythrocytes were stored at –50℃.

Determination of biochemical substances and enzymes. The spectrophotometry of thiobarbituric acid reactive substances (TBARS) was used to determine plasma LPO concentration and erythrocytic LPO concentration expressed as μmol/L and nmol/g • Hb respectively, while the spectrophotometry of inhibiting pyrogallol auto-oxidation was used to determine erythrocytic SOD activity expressed as U/g • Hb, and the spectrophotometry of coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocytic CAT activity expressed as K/g • Hb, and the improved Hafeman' s spectrophotometry was used to determine erythrocytic GPX activity expressed as U/mg • Hb[28,29], and the spectrophotometry of coloration of acetylcholine chloride-alkalescent hydroxylamine-ferric chloride was used to determine erythrocytic AChE activity expressed as U/g • Hb [30].

Chemtrue™ urine-MDMA one-step test kit was used *in vitro* for the diagnosis and confirmation of MDMA abusers with sensitivity of 500 ng/mL, which was made in Medyl Biotechnology, inc., San Diego, California, USA.

In determining the above biochemical substances and enzymes, the main analytical reagents, such as acetylcholinesterase, 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid, 1,2,3-trihydroxybenzene (pyrogallol), Cu/Zn-superoxide dismutase, catalase, were all purchased from SIGMA CHEMICAL COMPANY®, USA; and the other analytical reagents were all produced in China, and the fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. The main analytical instruments included HEWLETT PACKARD 8453- Spectrophotometer, USA, and UV-754-Spectrophotometer and 721-Spectrophotometer.

To avoid possible errors and bias, all experiments were standardized by using identical lot of all reagents and performed by the same laboratory assistants on the identical analytical apparatus[26-29].

Medical Statistic Analysis

All data in this study were statistically analyzed with SPSS11.0 for Windows software using a Compaq Pentium Ⅳ/1.6 G computer. The biochemical parameters in this study presented all normal distributions by Kolmogorov-Smirnov test, and were expressed as mean plus or minus standard deviation ($\bar{x} \pm s$) and 95 % confidence interval (95% CI). Hypothesis testing methods included independent-samples *t* test, c^2 test, reliability analysis and so on. In statistical analysis, the level of hypothesis testing (a) was $\leqslant 0.05$ so as to avoid false positives (-error), and the power of hypothesis testing (*power*) was $\geqslant 0.80$ to avoid false negatives (-error) [26-29].

RESULTS

Comparison of the Average Values ($\bar{x}\pm s$) of the Biochemical Parameters Between the MA Group and the HV Group

Compared with the HV group, the average values of LPO in plasma and erythrocytes in the MA group were significantly increased, while those of SOD, CAT, GPX and AChE in erythrocytes were significantly decreased (Table 1).

TABLE 1

Comparison of the Average Values ($\bar{x}\pm s$) of the Biochemical Parameters Between the MA Group and the HV Group

Group	*n*	Oxidative Constituents		Antioxidative Enzymes			Enzyme
		Plasma	Erythrocyte	Erythrocyte			Erythrocyte
		LPO (mol/L)	LPO (nmol/g ▪ Hb)	SOD (U/g ▪ Hb)	CAT (K/g ▪ Hb)	GPX (U/mg ▪ Hb)	AChE (U/g ▪ Hb)
MA	120	13.73±1.59 (13.45-14.02)	39.31±4.54 (38.48-40.13)	1838±146 (1812-1865)	248.6±65.2 (236.8-260.4)	20.30±5.74 (19.26-21.34)	204.3±53.3 (194.6-213.9)
HV	120	11.19±2.04 (10.82-11.56)	27.96±3.90 (27.25-28.66)	2126±165 (2096-2156)	309.2±81.4 (294.5-323.9)	29.10±7.89 (27.68-30.53)	314.6±68.7 (302.1-327.0)
*t**		10.771	20.771	14.319	6.363	9.884	13.891
P		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note. *Independent samples t test. Figures in parenthesis are 95 % confidence interval.

95% Confidence Interval of the Average Values ($\bar{x}\pm s$) of the Biochemical Parameters in the MA Group and the HV Group

The lower limits of 95% confidence interval (95% CI) of the average values of LPO in plasma and erythrocytes in the MA group were greater than the upper limits of 95% CI of the same average values in the HV group. The upper limits of 95% CI of the average values of SOD, CAT, GPX and AChE in erythrocytes in the MA group were less than the lower limits of 95% CI of the same average values in the HV group. And there was no superposition in 95% CI of the above biochemical parameters between the MA group and the HV group (Table 1).

Pearson Product-Moment Correlation Analysis Between the Value of AChE in Erythrocytes and the Values of the Biochemical Parameters for 120 MDMA Abusers

The findings of Pearson product-moment correlation analysis between the value of AChE in erythrocytes and the biochemical parameters for 120 MDMA abusers suggested that the values of LPO in plasma and erythrocytes were gradually increased with the decrease of the value of AChE in erythrocytes, and that those of SOD, CAT and GPX in erythrocytes were gradually decreased with the decrease of that of AChE in erythrocytes (Table 2).

TABLE 2

The Linear Regression and Correlation Between the Erythrocytic AChE Value and the Biochemical Parameters for 120 MDMA Abusers

Item	*n*	Regression	*r*	*P*[a]
Erythrocytic AChE with plasma LPO	120	$Y = 15.8110 - 0.0102\,X$	– 0.3414	<0.0005
Erythrocytic AChE with erythrocytic LPO	120	$Y = 48.1182 - 0.0431\,X$	– 0.5063	<0.0001
Erythrocytic AChE with erythrocytic SOD	120	$Y = 1537.89 + 1.4696\,X$	0.5351	<0.0001
Erythrocytic AChE with erythrocytic CAT	120	$Y = 102.477 + 0.7156\,X$	0.5848	<0.0001
Erythrocytic AChE with erythrocytic GPX	120	$Y = 7.6234 + 0.0621\,X$	0.5764	<0.0001

Note. [a] Linear regression and Pearson product-moment correlation analysis.

Reliability Analysis for the Biochemical Parameters Used to Estimate Oxidative Damage of the MDMA Abusers

The results of reliability analysis for the values of LPO in plasma and erythrocytes as well as those of SOD, CAT and GPX in erythrocytes reflecting the oxidative and lipoperoxidative damage of the MDMA abusers were as follows: the reliability coefficient (alpha) = 0.8124, the standardized item alpha = 0.9453. Single measure intraclass correlation was equal to 0.3022, with its 95% CI from 0.2386 to 0.3780, while average measure intraclass correlation amounted to 0.8124, with its 95% CI from 0.7581 to 0.8587 (F = 5.3304, $P < 0.0001$).

DISCUSSION

Lipoperoxide (LPO) is a product of peroxidation (auto-oxidation) of lipids that are exposed to oxygen, and lipoperoxidation is a source of free radicals and may be a cause of cancer, inflammatory diseases, atherosclerosis, aging, etc[6-8,11,16,28-44]. LPO and its metabolites, such as malondialdehyde (MDA), conjugated diene (CD) and others, are important poisonous residual products, significantly increased LPO, MDA and CD in human bodies may strongly attack DNA, proteins, enzymes, biological membranes, polyunsaturated fatty acids (PUFAs) and others, leading to lipoperoxidative damage of the membranes, and cytoclasis[6-8,11,16,28-44]. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH) are the most important antioxidases in the human bodies, and they play important roles in scavenging oxygen free radicals, such as superoxide anion radical, hydroxyl radical, hydroperoxyl radical and other free radicals as well as singlet oxygen, hydrogen peroxide and other reactive oxygen species which are excessive in the human bodies, preventing physiological and pathological aggravation of a series of free radical chain reactions induced by excessive superoxide anion radical, protecting the biological membranes against oxidative and lipoperoxidative damages[28-35,37-40,43,44]. The metabolic state and functional status of LPO and antioxidases in the human bodies are closely related with human health[14-26]. If abnormality occurs in their metabolism, the dynamic balance between the oxidative and antioxidative systems in the human bodies would be affected or destroyed[1, 6-8, 11, 16, 28-44]. As a consequence, a series of free radical chain reactions may be pathologically aggravated, thus inducing various diseases associated with the abnormal free radical reactions[1, 6-8, 11,16, 28-44]. Acetylcholinesterase (AChE) is one of the main biochemical indexes reflecting

neurotoxicity in the human bodies[30, 45, 46]. Marked decrease of AChE activity, especially erythrocytic AChE activity, can sufficiently suggest neurotoxicity in human brain and central nervous system[30, 45, 46].

The findings in the present study showed that in MDMA abusers the dynamic balance between oxidation and antioxidation was badly destroyed, and the oxidative stress resulted in pathological aggravation, thereby leading to severe oxidative and lipoperoxidative damages to MDMA abusers. There might be several interpretations.

The hyperthermia elicited by MDMA-abuse is a potentially deleterious condition that aggravates its direct toxic effects[6,7, 11-13, 15,16]. The hyperthermia *per se* is an important reason why a large number of excessive free radicals, such as superoxide anion radical, hydroxyl radical and others, are generated. MDMA-induced depletion of glutathione is potentiated, and the oxidative stress is aggravated, thus producing lipoperoxidation of PUFAs in cell membranes, increasing the contents of LPO, malondialdehyde, conjugated diene and others, decreasing the content of glutathione, and causing oxidative and lipoperoxidative damages of cells as well as loss of cell viability in human liver and hippocampus, striatum and cortex of the brain[6,7,11-13,15,16]. Metabolization of MDMA and auto-oxidation of MDMA metabolites form a large number of free radicals[11,16]. The reactions of xanthine/xanthine oxidase system, and Haber-Weiss reaction conducted and/or catalyzed by xanthine/xanthine oxidase with iron ion generate excessive hydroxyl radical and others[5]. Therefore, these physiological, pathological and biochemical reactions induced by MDMA and its metabolites are the main reasons why a large number of free radicals are generated in the bodies of MDMA abusers[5-7,11-13,15,16]. Especially while MDMA is abused, continually and chronically, formation of free radicals, lipoperoxide, malondialdehyde, conjugated diene and others can be maximized in the bodies of MDMA abusers[8]. In general, MDMA induced the following sequence of events resulting in serotonergic neurotoxicity and excessive free radicals formation: MDMA induced an acute release of 5-hydroxytryptamine (5-HT) and dopamine (DA), which was followed by depletion of intraneuronal 5-HT stores, the initially released 5-HT activated post-synaptic 5-HT2A/2C receptors located on gamma-aminobutyric acid (GABA) interneurons resulting in a decrease in GABAegic transmission and increased DA release and synthesis, the excessive DA release then might be transported into the depleted 5-HT terminal, and the excessive DA was then deaminated by monoamine oxidase B (MAO-B) located within the 5-HT terminal, thereby resulting in the generation of a large number of free radicals and reactive oxygen species, leading to lipoperoxidation and other oxidative stress in cellular membranes as well as oxidative and lipoperoxidative damages[8,9,12-14,23,25]. As a result, oxidative and lipoperoxidative damages occurred when endogenous free radical scavenging mechanisms became overwhelmed or exhausted[13, 28-46].

Long and high dose abuse of MDMA can lead to a series of digestive-systemic toxic reactions, such as anorexia or loss of appetite, nausea, vomit and diarrhea, etc., which result in the significant decrease of anti-vitamins (vitamin C, vitamin E and -carotene that are good scavengers of excessive free radicals) taken by MDMA abusers, thereby accelerating the aggravation of a series of free radicals chain reactions in the bodies of MDMA abusers [28-33,35,37,38,43,44]. Long and high dose abuse of MDMA may also produce circulatory- systemic toxic symptoms, such as cardio palmus, arrhythmia, and elevation or fall of blood pressure, as well as neurotoxic symptoms, such as dysphoria, anxiety, tension, depression, tremor and vertigo, so much as swoon, collapse, vague mind, mania, and so on. These toxic reactions and symptoms might promote the generation and release of excessive free radicals by means of reaction or catalysis 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, thereby leading to a significant increase of LPO levels in plasma

and erythrocytes as well as a significant decrease of activities of SOD, CAT and GPX in erythrocytes in MDMA abusers[28, 30, 35, 37, 43].

The consequence of the significant decrease of average values of erythrocytic AChE activities in MDMA abusers was found for the first time in the present study, and its main causes might be as follows. After it was digested in the alimentary tract and stomach, MDMA was rapidly combined with hydrochloric acid and other organic acid in the stomach to produce chemical and biochemical reactions, thus producing and releasing a large number of FRs and ROS, promoting violent aggravation of a series of FRs chain reactions, and then attacking strongly erythrocytic membranes with blood circulation, which formed biochemical poisoning reactions induced by excessive FRs and ROS, and resulted in oxidative and lipoperoxidative damages of erythrocytes and their membranes[30,35,37]. Subsequently, the functions of erythrocytes and their membranes were destroyed, and erythrocytic AChE activities in MDMA abusers were significantly decreased[30, 35, 37]. This fact showed that MDMA abuse also induced another neurotoxicity which inactivated acetylcholinesterase.

The findings in Pearson product-moment correlation analysis between the erythrocytic AChE activity reflecting sensitive and accurate toxicity induced by MDMA and the biochemical parameters suggested that the active groups in molecular structures of AChE were attacked and destroyed not only by MDMA and its metabolites, but also by a large number of FRs and ROS, and that the marked decrease and/or loss of the erythrocytic AChE activity occurred in the bodies of MDMA abusers[30].

In conclusion, the findings in the present study suggest that MDMA-abuse can induce another neurotoxicity that acetylcholinesterase activity is significantly inhibited, and that MDMA-abuse can aggravate a series of free radical chain reactions and oxidative stress in the bodies of MDMA abusers, thereby resulting in severe neural, oxidative and lipoperoxidative damages to them.

REFERENCES

1. Shankaran, M., Yamamoto, B. K., and Gudelsky, G. A. (2001). Ascorbic acid prevents 3,4- methylenedioxymethamphetamine (MDMA)-induced hydroxyl radical formation and the behavioral and neurochemical consequences of the depletion of brain 5-HT. *Synapse* **40**, 55-64.
2. Cadet, J. L., Thiriet, N., and Jayanthi, S. (2001). Involvement of free radicals in MDMA-induced neurotoxicity in mice. *Ann. Med. Interne.* **152**, Suppl. 3, IS 57-59.
3. Cadet, J. L., Ladenheim, B., Hirata, H., Rothman, R. B., Ali, S., Carlson, E., Epstein, C., and Moran, T. H. (1995). Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): evidence from using CuZn-superoxide dismutase transgenic mice. *Synapse* **21**, 169-176.
4. Cerruti, C., Sheng, P., Ladenheim, B., Epstein, C. J., and Cadet, J. L. (1995). Involvement of oxidative and L-arginine-NO pathways in the neurotoxicity of drugs of abuse *in vitro*. *Clin. Exp. Pharmacol. Physiol.* **22**, 381-382.
5. Kumagai, Y,. Lin, L. Y., Schmitz, D. A., and Cho, A. K. (1991). Hydroxyl radical mediated demethylenation of (methylenedioxy)phenyl compounds. *Chem. Res. Toxicol.* **4**, 330-334.
6. Carvalho, M., Carvalho, F., and Bastos, M. L. (2001). Is hyperthermia the triggering factor for hepatotoxicity induced by 3,4-methylenedioxymethamphetamine (ecstasy)? An *in vitro* study using freshly isolated mouse hepatocytes. *Arch. Toxicol.* **74**, 789-793.
7. Beitia, G., Cobreros, A., Sainz, L., and Cenarruzabeitia, E. (2000). Ecstasy-induced toxicity in rat liver. *Liver* **20**, 8-15.
8. Sprague, J. E. and Nichols, D. E. (1995). The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. *J. Pharmacol. Exp. Ther.* **273**, 667-673.
9. Esteban, B., O' Shea, E., Camarero, J., Sanchez, V., Green, A. R., and Colado, M. I. (2001). 3,4-Methylenedioxymethamphetamine induces monoamine release, but not toxicity, when administered centrally at a concentration occurring following a peripherally injected neurotoxic dose. *Psychopharmacology (Berl)* **154**, 251-260.
10. Aguirre, N., Barrionuevo, M., Ramirez, M. J., Del-Rio, J., and Lasheras, B. (1999). Alpha-lipoic acid prevents

3,4-methylenedioxy-methamphetamine (MDMA)-induced neurotoxicity. *Neuroreport* **10**, 3675- 3680.
11. Colado, M. I., O' Shea, E., Esteban, B., Granados, R., and Green, A. R. (1999). *In vivo* evidence against clomethiazole being neuroprotective against MDMA ('ecstasy')-induced degeneration of rat brain 5-HT nerve terminals by a free radical scavenging mechanism. *Neuropharmacology* **38**. 307-314.
12. Colado, M. I., O' Shea, E., Granados, R., Esteban, B., Martin, A. B., and Green, A. R. (1999). Studies on the role of dopamine in the degeneration of 5-HT nerve endings in the brain of Dark Agouti rats following 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') administration. *Br. J. Pharmacol.* **126**, 911-924.
13. O' Shea, E., Granados, R., Esteban, B., Colado, M. I., and Green, A. R. (1998). The relationship between the degree of neurodegeneration of rat brain 5-HT nerve terminals and the dose and frequency of administration of MDMA ('ecstasy'). *Neuropharmacology* **37**, 919-926.
14. Sprague, J. E., Everman, S. L., and Nichols, D. E. (1998). An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. *Neurotoxicology* **19**, 427-441.
15. Yeh, S. Y. (1997). Effects of salicylate on 3,4-methylenedioxymethamphetamine (MDMA)-induced neurotoxicity in rats. *Pharmacol. Biochem. Behav.* **58**, 701-708.
16. Colado, M. I., O' Shea, E., Granados, R., Misra, A., Murray, T. K., and Green, A. R. (1997). A study of the neurotoxic effect of MDMA ('ecstasy') on 5-HT neurones in the brains of mothers and neonates following administration of the drug during pregnancy. *Br. J. Pharmacol.* **121,** 827-833.
17. Colado, M. I., O' Shea, E., Granados, R., Murray, T. K., and Green, A. R. (1997). *In vivo* evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ('ecstasy') and p-chloroamphetamine but not the degeneration following fenfluramine. *Br. J. Pharmacol.* **121**, 889-900.
18. Colado, M. I. and Green, A. R. (1995). The spin trap reagent alpha-phenyl-N-tert-butyl nitrone prevents 'ecstasy'-induced neurodegeneration of 5-hydroxytryptamine neurones. *Eur. J. Pharmacol.* **280**, 343-346.
19. Cadet, J. L., Ladenheim, B., Baum, I., Carlson, E., and Epstein, C. (1994). CuZn-superoxide dismutase (CuZnSOD) transgenic mice show resistance to the lethal effects of methylenedioxya-mphetamine (MDA) and of methylenedioxymethamphetamine (MDMA). *Brain. Res.* **655,** 259-262.
20. Snodgrass, W. R. (1998). Clinical toxicology. In *Casarett & Doull's Toxicology – the Basic Science of Poisons* (Klaassen, C. D., Amdur, M. O., and Doull, J. Eds., 5th ed.), pp. 969-986. New York, McGraw-Hill Health Professions Division Press.
21. Ramos, K. S., Chacon, E., and Acosta, D. (1998). Toxic responses of the heart and vascular systems. In *Casarett & Doull's Toxicology – the Basic Science of Poisons* (Klaassen, C. D., Amdur, M. O., and Doull, J. Eds., 5th ed.), pp. 487-527. New York, McGraw-Hill Health Professions Division Press.
22. Thomas, J. A. (1998). Toxic responses of the reproductive system. In *Casarett & Doull's Toxicology – the Basic Science of Poisons* (Klaassen, C. D., Amdur, M. O., and Doull, J. Eds., 5th ed.), pp. 547-581. New York, McGraw-Hill Health Professions Division Press.
23. Gregus, Z. and Klaassen, C. D. (1998). Mechanisms of toxicity. In *Casarett & Doull's Toxicology – the Basic Science of Poisons* (Klaassen, C. D., Amdur, M. O., and Doull, J. Eds., 5th ed.), pp. 35-74. New York, McGraw-Hill Health Professions Division Press.
24. Parkinson, A. (1998). Biotransformation of xenobiotics. In *Casarett & Doull's Toxicology – the Basic Science of Poisons* (Klaassen, C. D., Amdur, M. O., and Doull, J. Eds., 5th ed.), pp. 113-186. New York, McGraw- Hill Health Professions Division Press.
25. Braitberg, G. and Kunkel, D. B. (1998). Amphetamines and amphetamine-like drugs. In *Emergency Medicine - a Comprehensive Study Guide* (Tintinalli, J. E., Ruiz, E., and Krome, R. L. Eds., 4th ed.), pp. 778- 780. New York, McGraw-Hill Health Professions Division Press.
26. Lang, T. A. and Secic, M. (1997). *How to Report Statistics in Medicine* (1st ed.), pp. 65-80. Philadelphia, Port City Press.
27. Lwanga, S. K., Tye, C. H., and Ayeni, O. (1999). *Teaching Health Statistics* (2nd ed.), pp. 3-102. World Health Organization, Geneva.
28. Zhou, J. F., Yan, X. F., Guo, F. Z., Sun, N. Y., Qian, Z. J., and Ding, D. Y. (2000). Effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress. *Biomed. Environ. Sci*. **13**, 44-55.
29. Zhou, J. F., Cai, D., Zhu, Y. G., Yang, J. L., Peng, C. H., and Yu, Y. H. (2000). A study on relationship of nitric oxide, oxidation, peroxidation, lipoperoxidation with chronic cholecystitis. *World J. Gastroenterology* **6**, 501-507.
30. Zhou, J. F., Xu, G. B., and Fang, W. J. (2002). Relationship between acute organophosphorus pesticide poisoning and oxidative damages induced by free radicals. *Biomed. Environ. Sci*. **15**, 177-186.
31. Xie, B., Zhou, J. F., Lu, Q., Li, C. J., and Chen, P. (2002). Oxidative stress in patients with acute coxsackie virus myocarditis. *Biomed. Environ. Sci*. **15**, 48-57.
32. Chen, P. and Zhou J. F. (2001). Abnormal metabolism of nitric oxide, oxidative stress and lipoperoxidative stress in patients with acute viral myocarditis. *Chinese Medical Journal* **114**, 1132-1135.
33. Chen, P. and Zhou, J. F. (2001). Changes of oxidative and lipoperoxidative metabolism in patients with acute viral myocarditis. *Journal of Zhejiang University (SCIENCE)* **2,** 448-452.

34. Mayes, P. A. (2000). Biologic oxidation. In *Harper's Biochemistry* (Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwell, V. W. Eds., 25th ed.), pp. 130-136. New York, McGraw-Hill Press.
35. Zhou, J. F., Yan, X. F., Ruan, Z. R., Peng, F. Y., Cai, D., Yuan, H., Sun, L., Ding, D. Y., and Xu, S. S. (2000). Heroin abuse and nitric oxide, oxidation, peroxidation, lipoperoxidation. *Biomed. Environ. Sci*. **13**, 131-139.
36. Mayes, P. A. (2000). Lipids of physiologic significance. In *Harper's Biochemistry* (Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwell, V. W. Eds., 25th ed.), pp. 160-171. New York, McGraw-Hill Press.
37. Zhou, J. F., Si, P. L., Ruan, Z. R., Ma, S. H., Yuan, H., Peng, F. Y., Sun, L., Ding, D. Y., and Xu, S. S. (2001). Primary studies on heroin abuse and injury induced by oxidation and lipoperoxidation. *Chinese Medical Journal* **114,** 297-302.
38. Zhou, J. F., Chen, P., Yang, J. L., Zhu, Y. G., Peng, C. H., and Wu, Y. L. (2000). Oxidative stress before and after operation in patients with chronic cholecystitis containing gallstone. *Biomed. Environ. Sci*. **13**, 254-262.
39. Murray, R. K. (2000). Muscle and the cytoskeleton. In *Harper's Biochemistry* (Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwell, V. W. Eds., 25th ed.), pp. 715-736. New York, McGraw-Hill Press.
40. McKee, T. and McKee, J. R. (2000). *Biochemistry: An introduction* (2nd ed.), pp. 289-330. New York, McGraw-Hill Press.
41. Lingappa, V. R. and Farey, K. (2000). *Physiological Medicine: A Clinical Approach to Basic Medical Physiology* (1st ed.), pp. 25-30. New York, McGraw-Hill Press.
42. Thuraisingham, R. C. and Raine, A. E. G. (1998). Endocrine manifestations of systemic disease: the heart. In *Clinical Endocrinology* (Grossman, A. Eds., 2nd ed.), pp. 1077-1082. Victoria, Blackwell Science Ltd Press.
43. Zhou, J. F. and Chen, P. (2001). Studies on the oxidative stress in alcohol abusers in China. *Biomed. Environ. Sci*. **14**, 180-188.
44. Chen, H. H. and Zhou, J. F. (2001). Low cholesterol in erythrocyte membranes and high lipoperoxides in erythrocytes are the potential risk factors for cerebral hemorrhagic stroke in human. *Biomed. Environ. Sci*. **14**, 189-198.
45. Worek, F., Kirchner, T., Backer, M., and Szinicz, L. (1996). Reactivation by various oximes of human erythrocyte acetylcholinesterase inhibited by different organophosphorus compounds. *Arch. Toxicol*. **70**, 497-503.
46. Hahn, T., Ruhnke, M., and Luppa, H. (1991). Inhibition of acetylcholinesterase and butyrylcholinesterase by the organophosphorus insecticide methylparathion in the central nervous system of the golden hamster (Mesocricetus auratus). *Acta. Histochem*. **91**, 13-19.

(*Received June* 6, 2002 *Accepted November* 10, 2002)