ORIGINAL ARTICLE

Alterations in Oxidative Stress Status During Early Alcohol Withdrawal in Alcoholic Patients

*Ming-Chyi Huang,[1,2] Chun-Hsin Chen,[2,3] Fu-Chuo Peng,[4] Sheng-Hui Tang,[5] Chiao-Chicy Chen[1,2]**

Background/Purpose: Alcohol-induced oxidative stress is the result of the combined production of reactive oxygen species [ROS; e.g. malondialdehyde (MDA), an index of lipid peroxidation] and impairment of antioxidant defenses [e.g. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which are involved in the elimination of ROS]. Little is known about the oxidative stress markers among patients with alcohol dependence in Taiwan. This study aimed to investigate serial alterations of various oxidative stress markers during early detoxification in alcoholic patients.
Methods: We enrolled 121 inpatients who fulfilled the DSM-IV-TR criteria for alcohol dependence, and 19 healthy controls. Fasting serum MDA level and antioxidant activity, including SOD, CAT and GPX, were measured at baseline in both groups, and after 1 and 2 weeks of detoxification in alcoholic patients.
Results: MDA level in alcoholics was higher at baseline than in healthy controls. It decreased after 1 week of detoxification, and normalized at week 2. SOD and GPX activities remained significantly lower throughout the 2-week period. CAT activity in alcoholics was comparable to that in the controls at baseline, but decreased at week 1 of detoxification, and was significantly lower than that in the controls after 2 weeks. Moreover, baseline MDA level was correlated with baseline CAT activity in alcoholics; the magnitude of the decrease in MDA level was correlated with the decrease in CAT activity following the 1-week detoxification.
Conclusion: The findings suggest severe oxidative stress and weakened antioxidant activity in alcoholic patients, and limited changes in oxidative stress in the early stages of alcohol withdrawal. [*J Formos Med Assoc* 2009;108(7):560–569]

Key Words: alcoholism, alcohol detoxification, alcohol withdrawal, malondialdehyde, oxidative stress

Excessive alcohol consumption has been reported as one of the leading causes of mortality.[1,2] Alcohol affects organ systems as a result of generation of free radicals during the metabolism of ethanol. Alcohol must be metabolized, primarily in the liver, through alcohol dehydrogenase and acetaldehyde dehydrogenase. The other pathway for alcohol metabolism is ethanol-inducible cytochrome P450 2E1 (CYP2E1). This is normally a minor pathway, but may play an important role at high ethanol levels and in chronic alcoholics.[3,4]

CYP2E1-mediated metabolism induced by alcohol generates more toxic metabolites than the parent compounds, including reactive oxygen species (ROS; superoxide anion and hydrogen

[1]Department of Psychiatry, Taipei City Psychiatric Center, Taipei City Hospital, [2]Department of Psychiatry, School of Medicine, Taipei Medical University, [3]Department of Psychiatry, Taipei Medical University-Wan-Fang Hospital, [4]Institute of Toxicology, College of Medicine, National Taiwan University, and [5]Toxic Center, Tri-Service General Hospital, Taipei, Taiwan.

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***Correspondence to:** Professor Chiao-Chicy Chen, Department of Psychiatry, Taipei City Psychiatric Center, Taipei City Hospital, 309 Song-De Road, Xing-Yi District, Taipei 100, Taiwan.
E-mail: cchen@tpech.gov.tw

peroxide) as well as ethanol-derived (hydroxyethyl) free radicals.[5,6] These products cause DNA damage, generate protein adducts, and initiate lipid peroxidation. Lipid peroxidation reflects the interaction between oxygen molecules and polyunsaturated fatty acids, and induces oxidation of various breakdown products of the latter. Among these, malondialdehyde (MDA) is a reliable marker of oxidative damage.[6–8] However, to protect the body from the adverse effects of free radicals, there are several antioxidant defense mechanisms that are involved in the elimination of ROS, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). SOD catalyzes superoxide radicals to hydrogen peroxide and thus is critical for prevention of damage from further toxic oxidative radicals generated by alcohol consumption.[9] CAT and GPX are responsible for further metabolism of hydrogen peroxide to water and oxygen.

Many studies have investigated the effect of alcohol on oxidative stress in humans. In comparison to controls, in alcoholics, MDA levels increased significantly in cerebrospinal fluid (CSF) or peripheral blood.[10,11] Reductions in lipid peroxidation have been reported consistently following alcohol withdrawal, despite various markers being utilized in different studies.[12–14] In general, antioxidant activity is lower in chronic alcoholics than in control subjects. Several studies have found that SOD and GPX activities are reduced after 2–3 weeks of alcohol withdrawal compared with those at baseline,[12,15,16] while one study has demonstrated that the deficiency in SOD activity did not improve from day 9 to 33.[10] Erythrocyte CAT activity shows no difference between alcoholics and controls, or after abstinence for alcoholics.[16,17]

Glutamate over-excitation has long been implicated in the pathophysiology of alcohol withdrawal symptoms.[18–20] Oxidative stress and excessive activation of glutamate receptors represent sequential as well as interacting processes. Since alcohol withdrawal symptoms mostly subside after 1–2 weeks of detoxification, it is important to explore the oxidative status during early withdrawal. Therefore, the aim of the present study was to explore the serial alterations of various oxidative parameters simultaneously throughout 2 weeks of alcohol detoxification in alcoholic patients in Taiwan, and to confirm the previous conflicting results by using a very small sample size.[21] We also examined the correlation between oxidative markers with regard to baseline values or the extent of changes.

Subjects and Methods

Subjects

This study was approved by the Institutional Review Board of Taipei City Psychiatric Center (TCPC). Alcoholic patients who were scheduled to be admitted to the alcohol detoxification ward in TCPC received a routine complete medical examination. Those with known severe physical illnesses were referred instead to a medical ward (e.g. patients with severe liver cirrhosis, infection, or those with post-admission illness). Between August 2003 and July 2005, patients were subjected to a thorough interview by two psychiatrists to ascertain the fulfillment of DSM-IV-TR (*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, text revision) criteria for alcohol dependence. Eligible patients were not using illicit drugs, did not have chronic systemic diseases such as diabetes mellitus or complicated cardiovascular disease, did not show any clinical evidence of the above-mentioned illnesses, or severe mental disorders, such as schizophrenia, bipolar disorder, or major depressive disorder. The control group included healthy subjects without known physical or psychiatric illnesses, identified by interview, as well as the results of routine laboratory tests. They had not previously met the diagnostic criteria for alcohol abuse or dependence, nor had they consumed alcohol during the previous 3 months. Written informed consent was obtained after the nature of the study was explained to every subject.

In alcoholic patients, a history of alcohol consumption was gathered, including duration of

alcohol dependence and average daily consumption in the past month. Alcohol consumption was stopped abruptly at admission. Fixed-dose detoxification consisted of four doses of 2 mg lorazepam, with gradual tapering thereafter, and trazodone as required for sleep problems. In addition, oral thiamine supplementation (150 mg/day) was given routinely to every subject to prevent serious neurological complications.[22] The detoxification ward was a restricted environment and provided a service for 1–2 weeks. The duration of hospitalization was decided on the basis of clinical improvement of alcohol withdrawal symptoms. All patients received the same meals supplied by the hospital during their stay.

Biochemical analysis

Venous blood samples were obtained within 24 hours of hospital admission (baseline), and subsequently on day 8 (after 1 week of detoxification) and 14 (after 2 weeks of detoxification), for measurement of serum MDA level and activities of SOD, CAT and GPX, after an overnight fast. The blood samples were centrifuged at 1000*g* for 15 minutes and serum samples were stored at −80°C until analysis.

For determination of MDA levels, the procedure was adapted from that developed by Jentzsch et al.[23] First, 0.5 mL of serum from alcoholic patients or controls was mixed with 25 μL butylated hydroxytoluene (88 mg/mL absolute alcohol) in ethanol. Next, 2.5 μL of 0.05 M sulfuric acid was added and vortexed for 10 seconds, and left to stand for 10 minutes at room temperature. Next, 25 μL of 0.2% thiobarbituric acid was added and vortexed. The reaction mixture was incubated at 100°C for 30 minutes in a water bath. The tubes were placed on ice to stop the reaction. After standing at room temperature, thiobarbituric acid reactive substances were extracted with 3 mL *n*-butanol. The *n*-butanol phase was centrifuged at 10,000*g* for 5 minutes. The absorbance of the *n*-butanol extract phase was measured at 450–610 nm with a UV/VIS spectrophotometer (Model V-550; Jasco International Co. Ltd., Tokyo, Japan).

For determination of SOD activity, Cu/Zn SOD levels were measured by sandwich ELISA using commercial kits and standard protocols (Superoxide Dismutase ELISA kit; ALEXIS Corp., Lausen, Switzerland).[24] Ten microliters of serum from alcoholic patients or controls, 50 μL of anti-SOD–horseradish peroxidase (HRP) conjugate, and 100 μL of PBS (pH 7.4) were added simultaneously to the wells of a microtitration plate, which was coated with anti-SOD antibodies (5 μg/mL). The plate was then shaken for 15 minutes at room temperature. After washing the wells three times with washing buffer (PBS with 1% Tween 20, pH 7.4), tetramethylbenzidine substrate (Sigma, St Louis, MO, USA; 100 μL/well) was added, and the plate was incubated for 10 minutes at room temperature. The reaction was stopped by adding 100 μL stop solution (1 M phosphoric acid) per well. Absorbance values were read at 450 nm using an ELISA reader (Model MRX; Dynex Technologies Inc., Chantilly, VA, USA). For standard curve generation, a Cu/Zn-SOD standard was prepared. Serial fold dilutions (0.08, 0.16, 0.32, 0.63, 1.25, 2.0, and 2.5 ng/mL) of Cu/Zn-SOD standard yielded a good correlation of SOD activity (SOD activity = 0.965X + 0.027, where X = dilution factor of the Cu/Zn-SOD standard; $r^2 = 0.9966$; data not shown).

The CAT activity in the serum of alcoholic patients and control subjects was measured using a BIOXYTECH Catalase-520 kit (OXIS Research, Portland, OR, USA) according to the manufacturer's protocol.[25] Thirty microliters of serum from the alcoholic patients or control subjects was incubated with 500 mL of 10 mM hydrogen peroxide for 1 minute at 37°C, and the reaction was stopped by addition of 500 μL sodium azide solution. Aliquots (20 μL) of the reaction mixture were then incubated with HRP/chromogen reagent for 10 minutes. Optical densities of 520 nm absorbance were read using an ELISA reader (Model MCC/340; Labsystems Multiskan, Farnborough, UK). For standard curve generation, various amounts of the standard CAT were incubated and the second-order polynomial regression was observed (CAT activity,

$U/mL = 0.00008X^2 - 341.73X + 118.54$, where X = absorbance at 520 nm; $r^2 = 0.9965$; data not shown).

GPX was determined by the Glutathione Peroxidase Assay Kit (Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's protocol.[26] Twenty microliters of the serum from alcoholic patients or control subjects, or diluted GPX was added to 100 μL of assay buffer and 50 μL cosubstrate. Twenty microliters of cumene hydroperoxide was added to start the reaction. The plate was incubated in the dark on an orbital shaker for a few seconds. The absorbance at 340 nm was read once every minute using a microtiter plate reader (MCC/340; Labsystems Multiskan) to obtain at least five time points. The actual extinction coefficient for NADPH at 340 nm is 0.00622 μM/cm. One unit of activity is equal to conversion of 1 nmol NADPH to $NADP^+$ per minute at 25°C.

Statistical analysis

Data were expressed as mean ± standard deviation. Analysis of covariance (ANCOVA) controlling potential confounders, especially age, was used to test the differences of various indexes between alcoholic and control groups. We used repeated-measures analysis of variance (ANOVA), with Bonferroni correction for multiple comparisons, to determine the differences of various indexes before and after detoxification in the alcoholic group. Pearson's correlation was used to measure the correlation between numerical variables. A value of $p < 0.05$ was considered statistically significant. Analyses were carried out using SPSS version 12.0 (SPSS In., Chicago, IL, USA). Subjects with missing data were excluded from the analyses.

Results

Clinical characteristics

One hundred and twenty-one patients and 19 healthy control subjects were recruited (Table 1). The control group (age, 30.4 ± 10.4 years) was younger than the alcoholic group (age, 42.2 ± 9.0 years). The classical biological markers of chronic

Table 1. Demographic characteristics and biological parameters of alcoholic patients and controls

	Controls ($n = 19$)	Alcoholics ($n = 121$)	
Mean ± standard deviation age (yr)*	30.4 ± 10.4	42.2 ± 9.0	
Sex			
Male	11	113	
Female	8	8	
Duration of alcohol dependence (yr)	–	13.2 ± 8.5 ($n = 113$)	
Mean alcohol consumption (g/d ethanol)	–	216.6 ± 107.1 ($n = 114$)	
Cigarettes/d	–	23.1 ± 13.1 ($n = 110$)	
		Baseline	1-wk detoxification
AST (U/L)	16.8 ± 2.6	130.2 ± 114.7† ($n = 109$)	53.5 ± 51.8†‡ ($n = 90$)
ALT (U/L)	14.6 ± 5.3	56.9 ± 66.3† ($n = 117$)	66.6 ± 74.2†‡ ($n = 91$)
GGT (U/L)	24.2 ± 7.3	401.1 ± 559.9† ($n = 117$)	226.8 ± 281.2†‡ ($n = 91$)
Total bilirubin (mg/dL)	0.6 ± 0.2	1.1 ± 0.9† ($n = 108$)	0.6 ± 0.5‡ ($n = 91$)

**Student's t test, $p < 0.05$ between control subjects and alcoholics; †significantly different from control group: $p < 0.05$, analysis of covariance between case and control group with age as a covariate; ‡paired t test, $p < 0.05$ between baseline and 1-week detoxification. AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = γ-glutamyltransferase.*

Table 2. Expression of oxidative stress markers between control and alcoholic groups

Mean ± SD	Control (n = 19)	Alcoholic patients during detoxification				
		Baseline		1 wk		2 wk
		n = 39	n = 82*	n = 39	n = 60†	n = 39
MDA (μM)‡	4.0 ± 0.9	10.0 ± 6.4§	8.8 ± 7.4§	5.0 ± 2.7§‖	5.3 ± 2.6§	4.6 ± 2.3‖
SOD (ng/mL)	159.6 ± 105.3	29.3 ± 21.7§	30.0 ± 24.6§	39.6 ± 29.7§	30.8 ± 29.2§	31.8 ± 20.2§
CAT (U/mL)‡	7.6 ± 4.6	9.2 ± 7.1	9.2 ± 7.7	5.7 ± 6.0	5.9 ± 4.6	4.2 ± 3.6§‖
GPX (nmol/min/mL)	205.1 ± 44.7	122.9 ± 57.9§	109.5 ± 59.2§	129.9 ± 41.2§	115.6 ± 47.1§	104.9 ± 48.3§

**Patients who withdrew from the study during week 1 or 2; †patients who withdrew from the study during week 2; ‡F (2, 256) = 21.0 for MDA, and F (2, 76) = 9.0 for catalase: all p < 0.01, repeated-measures analysis of variance before and after detoxification using 39 patients who completed the study; §significantly different from the control group: p < 0.05, analysis of covariance between case and control groups with age as a covariate at baseline, 1-week detoxification, and 2-week detoxification; ‖significantly different from baseline: p < 0.05, with Bonferroni correction for multiple comparisons. SD = standard deviation; MDA = malondialdehyde; SOD = superoxide dismutase; CAT = catalase; GPX = glutathione peroxidase.*

alcoholism, γ-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin were significantly higher in alcoholic patients than in healthy controls. All these markers decreased significantly after 1 week of detoxification.

Twenty-two and 60 patients, respectively, withdrew from the study during the first and second week of detoxification. We used ANOVA to compare clinical and biological variables between patients completing the 2-week study (n = 39), and those who withdrew during the first (n = 22) and second (n = 60) week. There were no differences in mean age (F = 0.14, df = 2, 118, p = 0.87), duration of alcohol dependence (F = 0.75, df = 2, 110, p = 0.48), mean alcohol consumption (F = 0.40, df = 2, 114, p = 0.67), MDA (F = 3.0, df = 2, 118, p = 0.053), SOD (F = 0.72, df = 2, 118, p = 0.49), CAT (F = 0.14, df = 2, 118, p = 0.87), and GPX (F = 1.19, df = 2, 118, p = 0.31) at baseline among alcoholic patients who completed the 2-week study and those who dropped out. In total, 99 patients completed 1 week of detoxification and 39 completed 2 weeks. The low retention rate (81.2% and 32.8% for weeks 1 and 2, respectively) may be explained in part by intolerance to the restricted detoxification ward by some patients, especially those with antisocial behavior—a frequent feature in alcoholic patients. Meanwhile, since alcohol withdrawal symptoms generally subside within 1 week, patients who experienced clinical improvement usually requested to be discharged after 1 week. Besides, there were still a few patients who refused to undergo withdrawal of the third blood sample, although they stayed for 2 weeks. Therefore, only one third of recruited patients completed the 2-week study.

Expression of oxidative stress markers

There were no differences in baseline oxidative stress markers between patients completing the study (n = 39) and those withdrawing during the first or second week (n = 82). Similarly, no differences were found in 1-week oxidative stress markers between those completing the study (n = 39) and those withdrawing during the second week (n = 60). Table 2 displays the results of various oxidative stress markers in control and alcoholic subjects at different time points. As a result of significant differences in age, we first used age as a covariate in the comparison of oxidative parameters between controls and alcoholic subjects who completed 2 weeks detoxification (n = 39). Baseline serum MDA levels in the alcoholic group were significantly higher than in the control group, started to decrease after 1 week of detoxification, and normalized at the end of 2 weeks. SOD and GPX activities in alcoholics were maintained at a significantly lower level than those in the controls, and showed no restoration throughout the 2 weeks. CAT activity of alcoholics was not significantly higher than that of controls at baseline,

Table 3. Correlation between oxidative stress markers and liver function tests at baseline among alcoholic patients ($n = 121$)

	MDA	SOD	CAT	GPX	AST	ALT	GGT
MDA	1						
SOD	0.06	1					
CAT	0.29*	0.27*	1				
GPX	0.05	−0.26*	−0.02	1			
AST	0.46*	0.22†	0.52*	0.06	1		
ALT	0.22†	0.24*	0.46*	0.06	0.67*	1	
GGT	0.41*	0.06	0.31*	0.12	0.58*	0.30*	1

$^{*}p < 0.01$; $^{\dagger}p < 0.05$. MDA = malondialdehyde; SOD = superoxide dismutase; CAT = catalase; GPX = glutathione peroxidase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = γ-glutamyltransferase.

but it was depressed after 1 week of detoxification, with an even lower level (44% lower) at the end of 2 weeks detoxification. Since significant differences were also noted for AST, ALT, GGT and total bilirubin between alcoholic and control groups, we compared the differences in various oxidative parameters at baseline and after 1 week of detoxification, using age and laboratory variables as covariates. Surprisingly, while the differences remained the same for SOD, CAT and GPX activities, the significance disappeared for MDA level.

Baseline MDA level was not correlated significantly with clinical variables in alcoholic patients ($n = 121$), including age, smoking, age at start of alcohol dependence, mean alcohol consumption, and duration of alcohol dependence. We further examined the relationship between serum MDA level and various antioxidant indexes. Table 3 demonstrates the correlation between baseline oxidative stress markers and liver function variables. At baseline, serum MDA level was correlated positively with CAT activity ($r = 0.29$, $p < 0.01$), but not with SOD and GPX activities. In addition, the magnitude of the decrease in MDA was correlated positively with that of CAT activity after 1 week of detoxification ($r = 0.27$, $p < 0.01$), but not with SOD and GPX.

Fifteen patients were positive for hepatitis B surface antigen (HBsAg) and 85 were negative. Data were not available for the remaining 21 patients. None of the oxidative status markers showed significant differences between patients with and without HBsAg.

Discussion

The results of the present study, which were in accordance with our previous findings,[21] indicated that serum MDA level and activities of SOD and GPX in alcoholic patients were significantly different from those in healthy controls. In alcoholics, MDA level was significantly higher than in the controls and gradually normalized after 2 weeks of detoxification. Meanwhile, there was a marked and persistent decrease in SOD and GPX activities throughout the 2-week withdrawal period. CAT activity in alcoholics was not higher than in the controls at baseline, but decreased markedly thereafter, and was even lower than in the controls after 2 weeks detoxification. Notably, after controlling for liver function in the comparison between alcoholic and control groups at baseline and 1-week detoxification, the differences disappeared for MDA level, while there was no change for SOD, CAT and GPX activities. Although there were positive correlations between baseline MDA level and baseline CAT activity, and between magnitude of the decrease in MDA level and magnitude of the decrease in CAT activity after the 1-week detoxification, the correlation coefficients were small (0.29 and 0.27, respectively).

The enhanced lipid peroxidation in alcoholics was in agreement with previous reports. By analyzing CSF, Tsai et al observed persistently higher lipid peroxide levels after alcohol withdrawal.[10] Several studies have consistently found elevated MDA levels in the peripheral blood of

patients undergoing alcohol withdrawal.[11,12,21,27–29] Likewise, our study demonstrated significant elevation in MDA level in alcoholic patients. The level decreased gradually after 1 week of abstinence and appeared comparable to that of the control subjects after 2 weeks detoxification. Thus, it is suggested that prompt detoxification in alcohol-dependent patients leads to restoration of pro-oxidant stress. However, after controlling for liver function tests, MDA levels at baseline and after 1-week detoxification did not differ from those in the controls. It has been reported that serum MDA concentration increases with the severity of liver disease.[30] Besides, liver damage and lipid peroxidation are considered as closely connected processes.[31] This implies that the significant elevation of MDA level in alcoholic patients is associated with underlying liver impairment, which contributes to the difference in MDA expression. However, this point requires confirmation in future studies.

SOD is responsible for reducing superoxide to hydrogen peroxide, and is the chief cellular defense against ROS. The effects of chronic alcohol exposure on the cellular content or activity of SOD are controversial, with reports of increases, no change, or decreases, depending on the model, diet, amount, and time of alcohol consumption.[12,16,32] In our study, compared with controls, SOD activity in alcoholic patients appeared to be significantly reduced. This is in agreement with previous reports.[11,12,21,27–29] The significant difference between the two groups at baseline or after 1 week of detoxification remained, even after controlling for liver function values in statistical analysis. We suggest that the decrease in SOD activity is a distinct feature of chronic alcohol exposure. Additionally, depressed SOD activity persisted throughout 2 weeks of abstinence, with negligible fluctuation. Although this observation differed from some studies that have indicated an increase in SOD activity after alcohol withdrawal,[12,16] it was consistent with that of Tsai et al, who found a long-lasting reduction in SOD activity in the CSF of alcoholic patients on days 9 and 33 after alcohol withdrawal.[10] SOD is critical in the detoxification of superoxide, which can initiate and propagate free radical oxidation.[33] Generation of free radicals by alcohol can be depressed by the addition of SOD.[34] Consequently, superoxide is proposed as the primary source for other oxyradicals that result from alcohol exposure. SOD is most sensitive to and the primary defense against ROS damage,[33] thus, it is depleted readily and is incapable of prompt compensation when encountering heavy oxidative injury. Therefore, we suggest that chronic alcoholism is associated with attenuated SOD activity, which is not balanced in the short term.

CAT normally plays a minor role in alcohol metabolism. It defends against and warrants the presence of hydrogen peroxide and related free radicals. It has been reported that chronic alcohol ingestion may cause dose-dependent elevation of CAT activity in rat plasma.[35] In our study, baseline CAT activity in alcoholics was 21% higher (not statistically significant) than that in the controls, but started to decrease significantly after 1 week, and was 44% lower than in the controls at week 2 of detoxification. A previous study has shown that the increase in erythrocyte CAT before abstinence in alcoholics is not significantly different from controls.[16] The reasons for the lack of differential CAT activities between alcoholic and control groups remain unclear. CAT might increase in alcoholics to meet the metabolic need of chronic alcohol ingestion, and return to defective levels after 2 weeks of abstinence. Animal studies have shown that CYP2E1 induction by alcohol can initiate lipid peroxidation, and as a result, lead to excessive oxidative injury.[36] Overexpression of CYP2E1 in cells enhances CAT expression, which confers resistance against pro-oxidants.[37] These results lend support to our data that a positive correlation existed between MDA level and CAT activity, either at baseline or for the magnitude of the changes after 1-week detoxification. Despite the correlation being only modest and requiring confirmation, it provides an initial clinical observation that, for alcoholic patients, there is a trend towards CAT activity elevation to counteract, at least in part, lipid peroxidation.

GPX is another cellular defense for removal of hydrogen peroxide, and it was found to be significantly lower in alcoholics than in controls. As with SOD, the drop in GPX activity was persistent during the 2-week withdrawal period. These results were inconsistent with those in the literature. For example, Girre et al described a reduction in GPX activity in alcoholic patients, but the activity appeared normalized during abstinence.[15] In contrast, some studies have shown that the higher baseline GPX activity decreased significantly after alcohol withdrawal.[12,16] Even after we controlled for the potential impact of liver function, we still found that GPX activity decreased significantly in alcoholic patients, either at baseline or after 1-week detoxification. Chronic alcohol exposure depresses GPX activity.[38] The Km value of GPX for hydrogen peroxide is much lower than that for CAT. Hence, GPX has been postulated to be sensitive to oxidative stress, and responsible for degrading low levels of hydrogen peroxide physiologically, while CAT might function when cellular levels of hydrogen peroxide are increased.[37] In CYP2E1-expressing cells, which manifest higher lipid peroxidation, the two enzymes appear to compensate for each other in scavenging hydrogen peroxide, because GPX was decreased by 30%, while CAT was increased twofold.[37] These observations support our results that in subjects with increased levels of MDA, CAT activity was elevated, whereas GPX was reduced. We suggest that, apart from SOD activity, alcoholic patients have severe and prolonged deficiency in GPX activity that needs longer than 2 weeks of abstinence to improve. Whether there are differential abnormalities in SOD and/or GPX activities at different stages of severity of alcohol dependence remains to be resolved.

Our study had some limitations. First, the benzodiazepine administered for detoxification might have affected oxidative stress. Some researchers have reported that diazepam, but not clonazepam, inhibits *in vitro* superoxide generation from human neutrophils,[39] which implies that clonazepam reduces oxidative stress. However, there is a paucity of data on the effects of lorazepam on oxidative stress in humans. Second, we cannot completely exclude the effect of adjunctive treatment with thiamine supplementation and trazodone. Thiamine exhibits direct antioxidant capacity, and treatment with it may reverse some oxidative stress parameters in rats.[40,41] Since thiamine supplementation is mandatory for alcoholic patients to prevent neurological sequela, we cannot avoid its confounding effect on oxidative stress. In addition, an *in vitro* study has demonstrated that trazodone has no effect on oxidative phosphorylation in mitochondria.[42] Our investigations did not reveal significant differences in various oxidative stress indexes between those with and without trazodone treatment. Thus, the effect of trazodone (50 mg) on oxidative stress seems minimal. Third, selection bias such as a mismatched control group and loss of follow-up should also be taken into consideration. Furthermore, the control group was younger (30.4 ± 10.4 years) than the alcoholic group (42.2 ± 9.0 years). Using ANCOVA to control for the age effect, we found that the differences of oxidative stress indexes between the control and alcoholic groups remained significant. We speculate that the possibility of an age effect on oxidative stress in our study was limited. Fourth, the dietary habits of our participants were not monitored. Finally, because we followed the abstinent alcoholic patients for only 2 weeks, whether the defects in SOD and GPX persisted after prolonged abstinence remains to be clarified by a longer follow-up study.

In conclusion, the serial and simultaneous assessment of various oxidative indexes showed that there was marked oxidative stress in the alcoholic patients without severe liver disease, as demonstrated by enhanced lipid peroxidation and depressed antioxidative mechanisms. The attenuation of raised MDA level and lowering of CAT activity appeared as early as after 1-week detoxification. The persistent low activity of SOD and GPX, as well as suppressed CAT response, indicated that alcoholic patients did not scavenge free radicals as readily as controls. Intriguingly, our data indicated, possibly for the first

time, that the increased MDA levels may have been influenced by underlying liver abnormalities. These observations of excessive oxidative stress might link with multiple morbidity in alcoholic patients. The impact of sustained abstinence in chronic alcoholic patients needs to be further investigated.

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