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Citric acid reduces the decline in P300 amplitude induced by acute alcohol consumption in healthy adults*

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Abstract: Event-related potential (ERP) is a reliable neuroelectric measure of brain activity that helps to confirm the assessment of mental status and cognitive impairment. Many studies have reported that alcoholics show a significantly lower ERP P300 amplitude than the norm. In the present study, ERP P300 waves were measured to evaluate the effect of citric acid on cognitive function during excessive alcohol consumption in healthy adults. Five volunteers were selected through clinical interview, physical examination, and psychiatric assessment for participation in this study. In a double-blind placebo-controlled before-after design, each subject was treated with 5 ml/kg body weight alcohol, 5 ml/kg body weight alcohol and 1 mg citric acid, or a placebo on three separate occasions, one week apart. ERP P300, blood biochemical indicators, blood alcohol concentrations (BACs) and acetaldehyde concentrations were assessed. Repeated measure analysis of variance (ANOVA) with a within-subjects factor was used to evaluate differences in blood biochemical indicators, BACs, blood acetaldehyde concentrations, and ERP P300 in the three sessions of assessments. Several blood biochemical indicators showed significant differences between treatments, including the levels of cholinesterase (CHE), total bile acid (TBA), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and glycylproline dipeptidyl aminopeptidase (GPDA). BACs after consumption of alcohol alone or citric acid with alcohol were significantly higher compared to those after placebo treatment (P<0.05). There were no significant differences in blood acetaldehyde concentrations between the treatments. The P300 amplitudes on the frontal (Fz), central (Cz), and parietal (Pz) regions of the scalp after consumption of alcohol were significantly lower than those after consumption of the placebo or citric acid with alcohol (P<0.05), while there were no significant differences between the latter two treatments. The results of this study suggest that citric acid could reduce the decline in ERP P300 amplitude and cognitive ability induced by acute alcohol consumption. It may also affect some blood biochemical indicators, but the specific mechanisms need further research.

Key words: Ethanol, Citric acid, Event-related potential (ERP) P300, Cognitive function, Acetaldehyde
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1 Introduction

Alcohol has sedative and detrimental effects on both performance and cognitive functioning (Wester *et al*., 2010), contributing to neurodegeneration and cognitive impairment (Cippitelli *et al*., 2010). Acetaldehyde, the first metabolite of alcohol, has been suggested to be involved in many behavioral effects after ethanol administration (Correa *et al*., 2012).

Event-related potential (ERP) is a reliable neuroelectric measure of brain activity that helps to confirm the assessment of mental status and cognitive impairment (Polich *et al*., 1990). Endogenous

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components are defined as a function of the psychological factors thought to determine the occurrence of ERP P300 (Gaillard, 1988). The endogenous P300 components of the ERP can be elicited using an oddball paradigm and can be used as an index of the neural events that underlie the allocation of attentional resources to a target stimulus (Isreal *et al*., 1980).

ERP components have been observed generally to be depressed under the influence of alcohol, but there is considerable variability in the sensitivity of the different ERP components to the suppressant effects of alcohol (Jääskeläiinen *et al*., 1996). Many studies have reported that alcoholics show significantly lower ERP P300 amplitude than the norm (Cloninger, 1987; Pfefferbaum *et al*., 1987). Alcohol dosages, ranging from blood alcohol concentrations (BACs) of 0.02 to 0.10 mg/ml, have been found consistently to reduce the P300 amplitude; however, the effect on an increase in P300 latency has been less consistent (Oscar-Berman, 1987).

The changes in underlying brain activities during mild cognitive impairment have shown the metabolic abnormalities of different brain regions (Li *et al*., 2010). Isotope studies have been used to evaluate total respiratory carbon dioxide production, and also strongly suggest that during ethanol metabolism the activity of the citric acid cycle is inhibited, and that cerebral glucose utilization decreases. The sites of inhibition were identified as the citrate synthase and isocitrate dehydrogenase steps, and the citrate content was decreased (Williamson *et al*., 1969). On-line ^{31}P-nuclear magnetic resonance (NMR) spectroscopy showed that long-term exposure to ethanol reduces the levels of adenosine triphosphate (ATP) and phosphocreatine (Fonseca *et al*., 2001).

Citric acid is one of the main metabolites in cerebral carbohydrate metabolism. In this study, we investigated whether the consumption of citric acid can improve cognitive function during ethanol consumption. To test this hypothesis, the effects of citric acid on ERP P300 during ethanol consumption were investigated. The blood biochemical indicators of liver and kidney function, indicators of lipoprotein metabolism, blood glucose levels, blood ethanol and acetaldehyde concentrations, and ERP P300 were compared between placebo and alcohol treatments to explore the effects of ethanol on cognitive functioning.

2 Materials and methods

2.1 Participants

Five healthy Chinese volunteers whose average age was 43.5 years were recruited from the First Affiliated Hospital, School of Medicine, Zhejiang University, China. All subjects were evaluated by clinical interview, physical examination, and psychiatric assessment. Subjects were excluded if they (1) showed signs or had a family history of neurological or psychiatric diseases, including nervosity; (2) had a history of craniocerebral trauma; (3) had severe liver or kidney diseases or diabetes mellitus; (4) had hypertension, hypotension, coronary heart disease, pulmonary heart disease, congenital cardiovascular disease, cerebral arteriosclerosis or other diseases which can influence electroencephalograms; (5) were currently taking sedatives or psychoanaleptics or had a fever; and (6) had positive acoustic reflexes at sensible sound intensities less than 16 dB.

2.2 Drugs

Three kinds of beverage were made with different contents: the first was a placebo (sweet rice porridge); the second included a 5 ml/kg body weight dose of alcohol, prepared as a mixture of sweet rice porridge and a 38% (78-proof) spirit, which was a kind of fermented alcoholic beverage produced by Xinjiang Yilite Industry Co., Ltd., China; the third included a 5 ml/kg body weight dose of alcohol, along with 1 mg citric acid which was melted into the mixture of sweet rice porridge and 38% (78-proof) spirit.

2.3 Procedures

All subjects avoided alcoholic beverages, orange juice, and cigarette or recreational drug use for one week before the study. In a double-blind placebo-controlled before-after design, each subject was given one kind of beverage when one test session was carried out. All subjects consumed the test or placebo beverages in random sequence over the three time points (with an interval of a week for three consecutive weeks). Each beverage was made to a volume of 500 ml and consumed over 10 min at room temperature from an opaque bottle, through a stopper packed with spirit soaked cotton wool, to provide olfactory masking (Shin *et al*., 2006). The timetable for each evaluation session is shown in Table 1. The BACs were measured 60 min after drinking.

Table 1 Timeline for each evaluation session

Time	Activity or assessment
11:30	Arriving at lab and screening for recent alcohol consumption by breath measurement of BACs, orange juice, cigarette and recreational drug use by clinical interview
12:00	Dinner
13:00	Preparation for the examination of ERP P300
13:30	Baseline assessments of ERP
14:30	Test or placebo beverage intake over 10 min at room temperature

The BACs and blood acetaldehyde concentrations were measured using a GC5890 gas chromatograph produced by Analytical Instruments Co., Ltd., Nanjing, China. The blood biochemical indicators were measured with an Abbott Aeroset Chemistry Analyzer, and ERP examinations for P300 were performed during the three sessions (consuming the three kinds of beverage on three different time points).

2.4 P300 of event-related potential

A Denmark Keypoint myoelectric potential/evoked potential meter was used to measure the ERP P300. TDH-39P telephonics headphones were connected to a computer through a model 'Breakout box'—a biological stimulus connection module auditory output. Silver-plated electrodes were used. The electrodes were placed in position at scalp sites according to the international 10-20 system for electroencephalograph (EEG). Electrodes were placed at the frontal (Fz), central (Cz), and parietal (Pz) regions, where the amplitude and latency of P300 were measured. Linked ears were used as reference electrodes, and FPz served as the ground connection.

Target (20%: 4000 Hz, 90 dB) and non-target (80%: 1000 Hz, 80 dB) tones of stimuli were given through headphones at time intervals of 600 ms. One thousand tones were presented comprising eight hundred 1000-Hz tones and two hundred 4000-Hz tones, generated by computer in a random order. Inter-electrode impedances were maintained below 5 kΩ, and the rejection limit was ±40 mV.

Testing was performed in a dimly lit room while each subject sat in a comfortable chair. The subjects were instructed to listen for 'high' tones (4000 Hz), and to press a key when one was heard. The apparatus recorded their reaction time and accuracy automatically. Prior to the formal experiment being carried out, 100 pilot trials were performed. Instructions were given to ensure that: the tones could be discriminated by each subject; subjects knew which tones to count; every subject was relaxed; and the performance of appliances, such as the consistency of electrode impedance, was checked. After an EEG was elicited from the scalp, the two signs were overlapped through the Denmark Keypoint myoelectric potential/evoked potential meter. Artifacts and other noise were eliminated automatically, and average potentials were measured and displayed immediately. The average latencies and amplitudes of P300 were obtained 100 times for each subject during each trial.

2.5 Statistical analysis

Data are expressed as mean±standard error of the mean (SEM). All statistical results were computed using SPSS data analysis software (Version 16.0; SPSS Inc., Chicago, IL, USA). Repeated measure analysis of variance (ANOVA) with a within-subjects factor was used to evaluate differences in blood biochemical indicators, BACs, blood acetaldehyde concentrations and ERP P300 in the three sessions of assessments. Mean differences between each pair of sessions were compared using the least significant difference (LSD) test. A P-value of less than 0.05 was considered to be statistically significant for all analyses.

2.6 Ethics

All subjects gave their informed written consent to participate in the study. The procedures followed were in accordance with the ethical standards of the local ethics review boards and with the Helsinki Declaration of 1975, as revised in 1983.

3 Results

There were significant differences in cholinesterase (CHE), total bile acid (TBA), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and glycylproline dipeptidyl aminopeptidase (GPDA) concentrations among the three treatments. Blood TG and VLDL-C levels after taking citric acid with alcohol were significantly

higher than those after placebo treatment (P<0.05). However, TC and HDL-C levels after taking citric acid with alcohol were significantly lower than those after placebo treatment (P<0.05). LDL-C and GPDA levels after taking alcohol or taking citric acid with alcohol were significantly lower than those after taking the placebo (P<0.05). There were no significant differences between other biomedical indicators among the three treatments (Table 2).

The BACs after administration of alcohol or citric acid with alcohol were significantly higher than those after placebo treatment, but there were no significant differences between other treatment comparisons (Table 3). There were no significant differences in blood acetaldehyde concentrations. Maybe when blood was drawn, alcohol in the liver had not been metabolized adequately.

The P300 amplitudes at the Fz, Cz and Pz sites after alcohol consumption were significantly lower than those after placebo consumption (P<0.05). There were significant differences in P300 amplitude between treatments with and without taking citric acid during drinking. The values were significantly higher after drinking with taking citric acid compared to those after drinking without taking citric acid (P<0.05). However, there were no significant differences in the P300 latency at Fz, Cz, or Pz among the three treatments (Table 4).

Table 2 Repeated measure analysis (ANOVA) results of blood biochemical measurements in the three trials

Parameter	Measurement value$^{\Delta}$			F value	P value
	Placebo	Alcohol	Citric acid and alcohol		
TP (g/L)	71.08±4.99	70.52±3.92	67.10±5.93	1.295	0.326
Albumin (g/L)	46.48±3.35	44.34±2.27	44.04±1.93	3.415	0.085
Globulin (g/L)	24.60±2.50	26.18±3.93	23.06±4.30	0.727	0.513
A/G	1.90±0.16	1.72±0.29	1.96±0.34	0.770	0.495
ALT (U/L)	19.60±8.44	18.40±6.95	19.00±7.48	0.203	0.821
AST (U/L)	19.80±8.04	19.60±7.60	19.60±9.02	0.009	0.991
ALP (U/L)	53.20±6.14	51.20±7.60	52.20±8.32	1.395	0.302
CHE (μmol/L)	9082±611	8625±689	8376±704$^{\#*}$	7.951	0.013
TBA (μmol/L)	12.60±3.65	8.20±7.05	5.20±1.48$^{\#*}$	3.611	0.076
TB (μmol/L)	7.40±2.07	6.60±2.70	8.20±2.95	1.085	0.383
DBIL (μmol/L)	2.00±1.00	2.80±1.48	2.60±0.55	2.364	0.156
IBIL (μmol/L)	5.40±1.52	3.80±1.64	5.60±1.95	2.056	0.190
GGT (U/L)	31.00±22.10	28.00±16.45	28.00±18.34	1.034	0.398
Cr (μmol/L)	72.00±9.70	71.80±5.93	76.00±11.51	0.845	0.465
BUN (mmol/L)	5.40±0.36	5.59±0.94	6.05±1.10	1.063	0.390
UA (μmol/L)	336.00±84.21	356.40±73.71	370.20±67.28	2.120	0.183
TG (mmol/L)	1.54±0.76	2.36±1.45	2.28±0.68$^{\#}$	4.395	0.052
TC (mmol/L)	4.37±0.62	3.94±0.74	3.91±0.75$^{\#}$	4.535	0.048
HDL-C (mmol/L)	1.24±0.26	1.10±0.32	1.05±0.21$^{\#}$	6.256	0.023
LDL-C (mmol/L)	2.51±0.46	1.89±0.70$^{\#}$	2.03±0.71$^{\#}$	8.757	0.010
VLDL-C (mmol/L)	0.62±0.32	0.95±0.60	0.83±0.23$^{\#}$	3.344	0.088
FBG (mmol/L)	6.27±1.48	6.65±1.96	6.04±0.50	0.341	0.721
GPDA (U/L)	80.60±11.87	73.20±15.71$^{\#}$	75.60±13.37$^{\#}$	5.546	0.031

$^{\Delta}$ Values are expressed as mean±SEM (n=5). TP: total protein; A/G: albumin/globulin; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; CHE: cholinesterase; TBA: total bile acid; TB: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; GGT: glutamyl transpeptidase; Cr: creatinine; BUN: blood urine nitrogen; UA: uric acid; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; FBG: fasting blood-glucose; GPDA: glycylproline dipeptidyl aminopeptidase. $^{\#}$ Compared with placebo administration with a within-subjects factor, P<0.05; * Compared with alcohol administration with a within-subjects factor, P<0.05

Table 3 Repeated measure analysis (ANOVA) results of plasma alcohol and acetaldehyde in the three trials

Parameter	Concentration (mg/ml)$^{\Delta}$			F value	P value
	Placebo	Alcohol	Citric acid and alcohol		
Plasma alcohol	0.0071±0.0113	0.4256±0.2673$^{\#}$	0.3124±0.2312$^{\#}$	10.682	0.006
Plasma acetaldehyde	0.0010±0.0006	0.0214±0.0236	0.0204±0.0262	3.002	0.107

$^{\Delta}$ Values are expressed as mean±SEM (n=5). $^{\#}$ Compared with placebo administration with a within-subjects factor, P<0.05

Table 4 Repeated measure analysis (ANOVA) results of ERP P300 in the three trials

Parameter		ERP P300 measurement value$^{\Delta}$			*F* value	*P* value
		Placebo	Alcohol	Citric acid and alcohol		
Amplitude	Fz	6.873±4.857	2.243±2.049$^{\#}$	8.725±5.689*	7.277	0.016
	Cz	7.589±4.306	2.229±1.017$^{\#}$	7.477±6.415*	9.724	0.007
	Pz	7.844±4.720	2.346±1.434$^{\#}$	8.284±6.004*	6.963	0.018
Latency	Fz	354±28.2	389±41.9	355±38.0	1.047	0.395
	Cz	353±29.5	386±38.3	354±36.0	0.669	0.539
	Pz	351±28.6	387±40.5	351±35.2	0.359	0.709

$^{\Delta}$ Values are expressed as mean±SEM (n=5). Fz: frontal; Cz: central; Pz: parietal. $^{\#}$ Compared with placebo administration with a within-subjects factor, P<0.05; * Compared with alcohol administration with a within-subjects factor, P<0.05

4 Discussion

Studies evaluating the effects of citric acid on the nervous system in alcoholics are rare. Previous studies have revealed that alcohol can inhibit the citric acid cycle function and reduce ATP levels. For this reason, it can be supposed that the addition of intermediate metabolites of the citric acid cycle may alleviate the injury to the nervous system induced by alcohol, with respect to cognitive function. In the present study, we found that the P300 increased in amplitude after alcohol consumption accompanied by citric acid intake compared to a session of alcohol intake without citric acid. Increasing the quantity of intermediate metabolites of the citric acid cycle when alcohol is consumed may improve the overall function of the citric acid cycle and reduce the negative effects on cognitive function. However, the precise mechanism operating at the cellular level still needs further study. Although this study had a small sample size, the physiological basis and differences in cognitive function associated with supplemental citric acid will provide a basis for future randomized controlled trials with a larger and more diverse study group.

Citric acid is the metabolic intermediate of the tricarboxylic acid (TCA) cycle. Decreasing generation of ATP contributes to slower cell proliferation and cell death. Citrate has been identified as a major TCA cycle constituent preferentially released by astrocytes, and the citrate supply is a more efficient energy source than lactate for neurons to produce ATP. This is especially true in the hypoglycemic state because citric acid is an intermediate metabolite in the TCA cycle (Meshitsuka and Aremu, 2008).

In patients with decreased cognitive function, the ERP P300 is smaller and occurs later than in age-matched normal subjects. In alcoholics, the amplitude of P300 is reduced and its latency prolonged, making this the ideal physiological marker for our current study (Miyazato and Ogura, 1993). Patients with a long-term drinking history have severe neural lesions and tend to have a reduction in P300 amplitude (Barrett *et al.*, 1987). In the present study, all subjects consumed alcohol for a normal period of time and the results support previous work that has found a reduction in the amplitude of P300 after drinking. Despite this, there were no significant differences in the P300 latency in the current study population.

Alcohol is one of the most widely abused substances in the world (Zucker *et al.*, 2008). Excessive alcohol (ethanol) consumption can have a substantial systemic effect on the human body. The most notable physiological responses occur in the central nervous system (CNS). Because ethanol can cross the blood-brain barrier easily, alcohol can directly induce a depressing effect on neural structures. This in turn can set in motion a wide spectrum of effects at the neurophysiological, morphological and neuropsychological levels (Moselhy *et al.*, 2001).

Alcohol in acute doses causes general and progressive impairment, disorganisation, retardation, and depression of CNS functioning (Martin and Siddle, 2003). The effects of alcohol on the CNS are complex and the behavioural effects of alcohol are dependent on its concentration. Low doses of alcohol produce behavioral stimulation due to an increase in neuronal excitation that results from the suppression of the inhibitory mechanisms which mediate stimuli entering

the CNS. As the alcohol dose increases, CNS depression increases and overcomes the disinhibition, thereby impairing and slowing cognition. Although at low doses the effect of alcohol is not consistent, reaction time (RT) is generally slowed by the ingestion of alcohol indicating the slowing of mental processes (Liguori and Robinson, 2001; Martin and Siddle, 2003).

Ethanol is converted into acetyl-CoA by liver enzymes and shuttled into the TCA cycle to be oxidized eventually (Haorah *et al.*, 2008). Acetaldehyde, which is the first active metabolite of ethanol metabolized in the CNS through the actions of catalase, has locomotor stimulant properties when administered in the ventricular system of the brain or into specific brain nuclei (Correa *et al.*, 2009). When cerebral metabolism of ethanol into acetaldehyde is blocked by catalase inhibitors, or acetaldehyde is inactivated, there is a suppressive effect on the actions of ethanol (Correa *et al.*, 2008).

The decreased physiological activity of the brain caused by ethanol is through the activation of inhibitory neurons and the inhibition of excitatory neurons. Through this process, a variety of cognitive abilities are affected including memory, analytic ability, and attention. Signs of cognitive impairment may precede those of alcohol-related neurological disorders by more than ten years (Tuck and Jackson, 1991). Chronic alcoholics display impairment in visuospatial scanning, utilizing, and manipulating information from visual images and anterograde spatial memory (Bowden and McCarter, 1993). The variables measured in the present study reflect the underlying neurological and physiological changes that occur with acute alcohol consumption.

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