

Effect of acute alcoholism on hepatic enzymes and oxidation/antioxidation in rats

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BACKGROUND: Around the world more and more people suffer from acute alcoholism. The purpose of this study was to determine hepatic enzymes and oxidation/antioxidation in rats with acute alcoholism.

METHODS: Rats were randomly divided into three groups: control, low-dose alcohol, and high-dose alcohol. Each alcohol group ($n=12$) was intravenously infused with ethanol at a dose of 0.3 or 0.7 g/kg body weight respectively. The control group ($n=11$) was intravenously infused with normal saline at a dose of 0.5 g/kg body weight. Blood was collected for detection of hepatic enzymes and index of oxidation/antioxidation.

RESULTS: The ratio of AST to ALT was 2.44 ± 0.46 , 2.57 ± 0.60 and 3.03 ± 0.46 in the three groups, and the difference was significant between the control and high-dose alcohol groups ($P \leq 0.05$). No significant changes were observed in the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (Tp), albumin (Alb), alkaline phosphatase (ALP), cholinesterase (ChE), total bilirubin (TB), C-reactive protein (CRP) and amylase. The levels of serum nitric oxide (NO) in the 3 groups were 39.2 ± 73.25 mol/L, 42.30 ± 4.60 mol/L and 47.86 ± 4.66 mol/L, and significant difference was seen between the control group and the high-dose alcohol group ($P < 0.01$). No significant difference was found in the levels of serum superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), and CRP in the 3 groups.

CONCLUSION: The ratio of AST to ALT appears to be a useful index for acute alcohol intoxication. NO is involved in the mechanism of acute alcohol intoxication.

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KEY WORDS: alcoholism; infusion; nitric oxide; oxidation/antioxidation; animal model; hepatic enzyme

Introduction

Alcoholic liver disease (ALD) remains one of the most common causes of chronic liver disease. Excessive alcohol consumption can result in multiple organ injury, of which ALD is most common. ALD has a known etiology but a complex pathogenesis. Interactions between acetaldehyde, alcohol dehydrogenase, reactive oxygen and nitrogen species, inflammatory mediators, immunity and genetic factors appear to play prominent roles in the development of ALD.^[1-4] Around the world, more and more people suffer from acute alcoholism and the mortality of acute alcoholism is increasing. It is recognized that abuse of alcohol increases the risk of fatal injuries.^[5] Whether acute alcoholism shares common mechanism with chronic ALD remains obscure. The purpose of this study was to determine hepatic enzymes and oxidation/antioxidation in rats with acute alcoholism.

Methods

Male Sprague-Dawley rats weighing 450 and 550 g from the Experimental Animal Center of Medical College, Zhejiang University, Hangzhou, China were housed at a temperature of 23 °C to 25 °C and fed on a standard food with free access to drinking water. Thirty-five rats were randomly divided into three groups: control, low-dose alcohol, and high-dose alcohol. Each alcohol group ($n=12$) was intravenously infused with ethanol at a dose of 0.3 or 0.7 g/kg body weight in 5 minutes respectively.^[6-8] The control group ($n=11$) was intravenously infused with normal saline at a dose of 0.5 g/kg body weight. Blood samples were collected from the femoral vein after 4 hours, and then the rats were sacrificed. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (Tp), albumin (Alb), cholinesterase (ChE), alkaline phosphatase (ALP), total bilirubin (TB), nitric oxide (NO), superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), C-reactive protein (CRP) and amylase were detected.

The data were analyzed using SPSS 11.0 statistical package. Statistical significance of the difference was de-

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Table. Laboratory data of control and alcohol groups

	Control (n=11)	Low-dose alcohol (n=12)	High-dose alcohol (n=9)	P values
Tp (g/L)	76.14±1.88	76.57±4.01	75.11±3.66	0.608
Alb (g/L)	41.29±2.00	41.12±2.13	39.34±1.87	0.079
ALT (U/L)	74.27±33.17	73.58±11.70	81.67±23.29	0.717
AST (U/L)	182.82±101.68	186.33±38.07	251.56±99.84	0.319
AST/ALT	2.44±0.46	2.57±0.60	3.03±0.46*	0.044
ALP (U/L)	134.64±23.35	113.75±14.48	124.78±26.84	0.085
ChE (U/L)	126.45±28.26	165.33±47.13	159.56±38.33	0.056
TB (μmol/L)	30.82±19.59	25.00±14.10	31.11±22.01	0.681
CRP (g/L)	12.12±1.02	9.93±3.55	12.69±0.92	0.052
Amylase (U/L)	2697.45±498.88	2772.58±267.64	2797.11±214.26	0.801
NO (μmol/L)	39.27±3.25	42.30±4.60	47.86±4.66**	<0.001
GSH (mg/L)	210.02±36.11	229.12±27.77	229.22±41.60	0.351
SOD (NU/L)	177.59±7.24	175.83±3.80	168.61±13.68	0.070
MDA (nmol/L)	7.88±1.06	7.93±1.12	7.96±0.99	0.986

Data are expressed as means±SD. Compared with the control group, * : $P < 0.05$; ** : $P < 0.01$.

terminated by one-way ANOVA and a P value less than 0.05 was considered significant.

Results

No significant changes of biological behavior were observed in rats of the control and low-dose alcohol groups. Three rats in the high-dose alcohol group died suddenly. Dyspnea was found in all rats of the high-dose alcohol group after alcohol infusion.

The mean levels of ALT of the 3 groups were 74.27±33.17, 73.58±11.70 and 81.67±23.29 U/L, and those of AST were 182.82±101.68, 186.33±38.07 and 251.56±99.84 U/L, respectively. No significant difference was seen between the 3 groups. The ratio of AST to ALT was 2.44±0.46, 2.57±0.60 and 3.03±0.46 in the 3 groups, and the difference was significant between the control and high-dose alcohol groups ($P > 0.05$). Nevertheless, there were no significant changes in the levels of Tp, Alb, ALP, ChE, TB, CRP and amylase (Table).

The levels of serum NO were 39.27±3.25, 42.30±4.60 and 47.86±4.66 μmol/L. There was no significant difference between the control and low-dose alcohol groups ($P > 0.05$) in contrast to significant difference between the control and high-dose alcohol groups ($P = 0.000$). Nevertheless, no significant difference was found in the levels of serum SOD, GSH, MDA and CRP in the 3 groups.

Discussion

The diagnosis of ALD is dependent on the exclusion of other causes but a significant history of alcohol consumption.^[9,10] Hepatic transaminase levels are only modestly elevated and the ratio of AST to ALT appears to be a

useful index for distinguishing non-alcoholic steatohepatitis from ALD. Hepatic transaminase levels less than 1 suggest non-alcoholic steatohepatitis, and a ratio of greater or equal to 2 is strongly suggestive of ALD in humans.^[11,12] In this study, the ratio of AST/ALT was greater than 2, which indicates that difference may exist between humans and rats. The significant differences between the control and high-dose alcohol groups ($P < 0.05$) suggest that the elevation of the AST/ALT ratio be suggestive of acute alcoholism.

NO was thought to be the endothelium-derived relaxing factor synthesized from amino acid L-arginine.^[13] It has been reported that NO synthesis also occurs in the liver playing different biological roles. NO in the liver produced by cytokine-treated macrophages, endothelium and hepatocytes plays a vital role in host responses. The synthesis of NO is induced by many cytokines and endotoxin in the liver. It was reported that NO protects against cellular damage and cytotoxicity from reactive oxygen species^[14] and also inhibits hepatocyte Tp synthesis, gluconeogenesis and mitochondrial respiration.^[15] Moreover, NO is considered to be related to portal hypertension associated with a hyperdynamic circulation through regulating blood flow. NO seems to play an important role in the development of ALD.^[16,17] In this study, the concentration of NO elevated with the increased volume of alcohol infusion. These data suggest that acute alcoholism may be related to NO in the liver. But it is not well understood that NO as a mediator molecule plays a possible protective or injurious role in ALD. Report suggested that decreased NO production by nonparenchymal cells may contribute to liver injury in ethanol-fed rats, and that the compensatory increase of NO production in hepatocytes may contribute to centrilobular liver injury.^[18] Clemens^[19] reported that the most important factors in determining whether NO will be protective or injurious are the localization of NO

production, the amount of NO being produced, and the relative amounts of superoxide anion being produced in the same location as the NO.

It has been suggested that oxidative stress may play an important role in pathogenesis of ALD.^[20-22] Ethanol oxidation leads to formation of several free radical species in hepatocytes, including hydroxyethyl radical, superoxide anion, and hydroxyl radical. These ethanol-induced free radicals inflict oxidative damage on a wide range of intracellular compounds. Ethanol-induced radical formation is typically attributed to ethanol oxidation. Whether ethanol-induced sufficient free radical production to cause liver injury in humans remains uncertain, but alcohol consumption indeed leads to depletion of several antioxidants in the liver such as GSH and SOD. GSH as a tripeptide has a hydrosulfide group which often oxidizes to cystine and is the major component of nonprotein hydrosulfide. GSH can remove H₂O₂ and alkyl hydroperoxide (ROOH). When the cell is attacked, GSH can also combine competitively with foreign electrophilic subject with toxicity in order to keep the cell and its membrane integrated. When superoxide anion (O₂⁻) is catalyzed to O₂ and H₂O₂ by SOD, and H₂O₂ is further catalyzed to O₂, radical-induced lipid peroxide is prevented. The elevation of MDA indicates increased production of lipid peroxide so that the content of MDA could reflect the extent of the cell being attacked by free radical. In our study no significant difference was found in serum GSH, SOD and MDA in rats after acute alcohol infusion, suggesting that no overproduction of reactive oxygen species by mitochondria was found after acute alcohol infusion. It was reported that formation of lipid radicals was enhanced after acute alcohol administration.^[23]

CRP is a component of acute phase response to infection, inflammation, and trauma. A major activity of acute phase proteins is to limit the inflammatory response. CRP is acute-phase reactant that is usually present at a high concentration in the serum of patients with liver disease.^[24] IgA antibodies against fecal endotoxin have been found to be closely correlated with the plasma concentration of CRP in patients with ALD.^[25] But few studies have focused on CRP in patients with ALD. In the present study, the serum CRP levels were observed in rats after acute alcohol infusion but they were not increased in the alcohol group. Interestingly, the expression of CRP was upregulated in alcohol-induced acute liver injury.^[26] Whether the measurement of serum CRP is useful in assessing the activity of alcoholic hepatitis remains unknown.

Acute alcoholic pancreatitis may be one of the most serious adverse consequences of alcohol abuse. The pathogenesis of alcoholic pancreatitis is not fully defined. The ultimate picture is one of tissue autolysis by activated proteolytic enzymes. The triggers for such activation,

however, are not clear. A direct toxic-metabolic effect of ethanol on pancreatic acinar cells may be one of the theories. Serum amylase is a cornerstone laboratory test for the establishment of the diagnosis of acute pancreatitis because it is simple, available and highly sensitive. This study was to evaluate the level of amylase to prove whether acute alcohol infusion could lead to acute alcoholic pancreatitis. The level of amylase was not elevated in the animal model of acute alcohol infusion. Why no pancreatitis develops in alcohol intoxication is still obscure. Pancreatitis is clearly associated with alcohol abuse,^[27] but only a small population who abuse alcohol develops obvious pancreatitis. Pezzilli et al^[28] reported that the levels of serum amylase and pancreatic isoamylase were abnormally high in 16 of the 17 patients (94%), whereas the level of serum lipase was abnormally high in all patients with acute alcoholic pancreatitis. Some observations have drawn a conclusion that the development of alcoholic pancreatitis requires cofactors.^[29]

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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