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Effect of Protein and Fiber Levels on Ethanol-Induced Brain Damage in Rats

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Abstract

The purpose of this study was to investigate the effect of protein and dietary fiber levels on the activities of ethanol metabolizing enzymes of the brain in acute and chronic ethanol-treated rats. Male Sprague-Dawley rats were fed on diets containing two levels of protein (7%, 20%) with two levels of fiber (5%, 10%) for 5 weeks. Rats were orally administered 40% (v/v) ethanol (5 g/kg body weight) 90 min before decapitation in the acute ethanol-treated groups and 25% (v/v) ethanol (5 g/kg body weight) once a day for 5 weeks in the chronic ethanol-treated groups. Cytosolic alcohol dehydrogenase (ADH) activities were higher than those of mitochondrial ADH. The ADH activities were increased by 20% protein and 5 % fiber levels in the diet in two fractions, but were decreased by chronic ethanol treatment. Mitochondrial aldehyde dehydrogenase (ALDH) activities did not change by ethanol treatment but were increased by the 20% protein level. However, cytosolic ALDH activities were decreased by chronic ethanol treatment at the 5% fiber level and did not change with protein levels. Both ALDH activities were higher in the 10% fiber groups than the 5% fiber groups. Cytochrome P-450 contents were significantly increased in the chronic ethanol-treated groups but xanthine oxidase (XO) activities did not change. P-450 contents and XO activities were significantly decreased in both the low protein and fiber groups.

Key words: protein, dietary fiber, ethanol metabolizing enzyme system

INTRODUCTION

Alcoholism is the most common form of drug abuse in modern society. Unquestionably the liver plays a major role in the disposal of ethanol from the body; it alone accounts for more than 90% of total systemic metabolism of ethanol. Ethanol load taxes the biochemical capacities of the liver, and derangements in hepatic function have been attributed to local biochemical readjustments associated with the oxidation of ethanol to acetaldehyde and eventually to acetate (1). In view of its relatively low alcohol dehydrogenase activity, the contribution of the brain to the disposal of an ethanol load would be insignificant, but may play a fundamental role in the biochemical bases of the neural disorders associated with prolonged alcohol ingestion or withdrawal (2). The most dramatic pharmacological actions of ethanol are manifested in the central nervous system. The mechanism by which alcohol produces behavioral changes remains uncertain. Theories that have been proposed include a direct neurotransmitter role for ethanol; effect on cell membrane fluidity; toxic effects on oxidative metabolism; and toxic effects of acetaldehyde (3).

The toxic reaction of excess ethanol consumption may result from the reactivity of acetaldehyde. Like other aldehydes, acetaldehyde can condense with amino groups to form a Schiff base. Its reaction with the amino-terminal and lysine amino groups of proteins results in damage to the protein. Schiff base formation occurs in a number of pathological conditions (4). Nutritional disorders can be related to alcoholism in several ways, since ethanol abuse may alter metabolism, transport, utilizaton, activation and storage of almost every essential nutrient (5). In particular, large doses of ethanol appear to inhibit protein synthesis in the brain. Ethanol also affects ionic transport processes, adenine nucleotides, amino acid and protein metabolism (6). The metabolic consequence of such effects of ethanol have been criticized. That nutritional deficiencies and ethanol toxicity may act synergistically in the nervous system outlines the importance of adequate nutritional strategies in the treatment of alcoholism.

Pectin, one of the abundant fibers found in citrus fruit and apples, has gel-forming properties and is a hydrophilic polymer with carboxylic groups (7). This viscous property decreases the accessibility of protein molecules to the digestive enzymes and product of digestion (8). But studies on the interaction of ethanol, protein and fiber are rare.

Therefore in this study we attempted to investigate the effect of protein and dietary fiber levels on the activities of ethanol metabolizing enzymes of brain in acute and chronic ethanol-treated rats.

MATERIALS AND METHODS

Experimental animals and diets

This study was conducted with weanling male Sprague-

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Dawely rats. They were housed individually in stainless-steel cage in a temperature and light controlled room. After an acclimatization period of 10 days during which they were fed a AIN-76 (9) standard laboratory chow diet, they were assigned, at random, to one of eight experimental groups (Table 1). Their average body weights at this time were 100±10 g. They were fed on diets containing two levels of protein (7%, 20%) with two levels of fiber (5%, 10%) and given water *ad libitum* for 5 weeks. The rats were orally administered 40% (v/v) ethanol (5 g/kg body weight) 90 min before decapitation in the acute ethanol-treated groups and 25% (v/v) ethanol (5 g/kg body weight) once a day for 5 weeks in the chronic ethanol-treated groups.

Tissue preparations

Rats were killed by decapitation and their tissue were quickly excised, removed, and homogenized in 10 parts (w/v) of 10 mM potassium phosphate in 5 mM EDTA (pH 7.4) solution using a tissue homogenizer with a teflon pestle. The homogenate was centrifuged at 600×g for 10 min, and the supernatant was further centrifuged at 10,000×g for 20 min to yield a mitochondria pellet, and the supernatant was further centrifuged at 105,000×g for 60 min to yield a microsomal pellet and cytosol supernatant.

Biochemical assays

Alcohol dehydrogenase activity was measured by the Bergmeyer method (10). Aldehyde dehydrogenase activity was measured by the Koivula and Koivusalo method (11). Cytochrome P-450 content was measured by the Omura and Sato method (12,13). Xanthine oxidase activity was measured by the Stirpe and Della method (14). The amount of protein in the samples were measured according to the Lowry et al. method (15).

Table 1. Composition of experimental diets

Ingredients	Content (%)			
	LPNF	LPHF	NPNF	NPHF
Casein	7.0	7.0	20.0	20.0
Corn starch	63.0	58.0	50.0	45.0
Sucrose	15.0	15.0	15.0	15.0
Pectin	5.0	10.0	5.0	10.0
Corn oil	5.0	5.0	5.0	5.0
AIN-mineral mixture[1]	3.5	3.5	3.5	3.5
AIN-vitamin mixture[2]	1.0	1.0	1.0	1.0
DL-Methionine	0.3	0.3	0.3	0.3
Choline chloride	0.2	0.2	0.2	0.2

[1]Mineral mixture (g/kg) according to AIN-76

Calcium phosphate, dibasic	500.0	Zinc carbonate	1.6
Sodium chloride	74.0	Cupric carbonate	0.3
Potassium citrate, monohydrate	220.0	Potassium iodate	0.01
Potassium sulfate	52.0	Manganese carbonate	3.5
Magnesium oxide	24.0	Chromium potassium sulfate	0.55
Ferric citrate	6.0		
Sucrose	118.04		

[2]Vitamin mixture (g/kg) according to AIN-76

Thiamin-HCl	0.6	Biotin	0.02
Riboflavin	0.6	Cyanocobalamin	0.001
Pyridoxine-HCl	0.7	Retinyl acetate	0.8
Nicotinic acid	3.0	DL-tocopherol	3.8
Ca-panthothenate	1.6	7-dehydrocholesterol	0.0025
Folic acid	0.2	Menadione	0.005
Sucrose	988.67		

LPNF: Low protein, normal fiber diet group
LPHF: Low protein, high fiber diet group
NPNF: Normal protein, normal fiber diet group
NPHF: Normal protein, high fiber diet group

Statistical analyses

Data from individual experiment were expressed as the mean±S. E. All statistical analyses were performed on a SAS program and significant difference between treatment means were determined by using Duncan's multiple range test (16) that $p<0.05$ was judged to be statistically significant. The interaction of the ethanol, protein and fiber was evaluated by a two-way analysis of variance (17).

RESULTS AND DISCUSSION

Alcohol dehydrogenase activity

As shown in Table 2, the activities of alcohol dehydrogenase (ADH) in mitochondria were 42.1~52.4% of cytosol. The ANOVA analysis revealed the significant difference between acute and chronic ethanol treatment in mitochondria and cytosol. At the 20% protein (NP) level, the activities of chronic ethanol-treated groups were significantly lower than those of acute ethanol-treated groups in mitochondria, whereas the activities in cytosol did not differ siginificantly except in the NPNF group. The presence of ADH in the brain of mammals has been controversial. Raskin and Sokoloff (18) reported ADH acitivity in the rat brain. Buhler et al. (19) demonstrated the presence of ADH in both the cerebral hemispheres and cerebellum of the human brain. Beisswenger et al. (20) characterized ADH from the brain of the human, bovine and rat. Raskin and Sokoloff (1) reported that the pH optimum, kinetic properties and response to inhibitors in brain are similar to those of hepatic ADH. Thus, we thought that the properties of ethanol metabolizing enzyme in brain might be similar to those of the liver enzyme.

The activities of ADH were higher in the cytosol than in the mitochondria but activities by chronic ethanol treatment were lower in the cytosol than in the mitochondria. The ADH activities of mitochondria in the 7% protein (LP) groups were lower than those of the 20% protein (NP) groups both in acute and chronic ethanol-treated groups. Effect of fiber levels on ADH activity was not observed. Cytosolic ADH activity in acute ethanol-treated groups were also increased by 20% protein (NP) levels.

These results were similar to those reported by Wilson et al. (21) that nutritional deficiency may impair ethanol detoxification processes. Low protein diets were shown to reduce brain ADH activity and rate of ethanol metabolism in the rat (22). Thus, it seems conceivable that the effect of ethanol may be exacerbated in malnourished subjects, since a reduced rate of ethanol metabolism would result in higher and more

Table 2. Effect of protein and dietary fiber levels on brain ADH activity in rats with acute and chronic ethanol treatments

(n moles/min/mg protein)

Group[1)]	Mitochondria		Cytosol	
	Acute	Chronic	Acute	Chronic
LPNF	4.07 ± 0.38^{bc}	3.61 ± 0.41^{c}	9.27 ± 0.61^{bc}	9.02 ± 0.38^{bcd}
LPHF	3.85 ± 0.50^{bc}	3.48 ± 0.48^{c}	8.66 ± 0.83^{cd}	8.25 ± 0.79^{d}
NPNF	5.47 ± 0.40^{a}	4.39 ± 0.41^{b}	10.96 ± 0.70^{a}	9.87 ± 0.54^{b}
NPHF	5.13 ± 0.53^{a}	4.26 ± 0.28^{b}	9.78 ± 0.79^{b}	9.31 ± 0.63^{bc}
ANOVA	F		F	
Ethanol(A)	22.65^{**}		6.77^{*}	
Protein(B)	52.75^{**}		30.50^{**}	
Fiber(C)	2.04^{NS}		13.38^{**}	
A×B	3.62^{NS}		1.12^{*}	
A×C	0.28^{NS}		0.29^{NS}	
B×C	0.05^{NS}		0.19^{NS}	
A×B×C	0.03^{NS}		0.85^{NS}	

[1)]See the legend of Table 1

Acute ethanol-treated group: Rats were administered orally 40% (v/v) ethanol (5 g/kg body weight) before 90 min of decapitation

Chronic ethanol-treated group: Rats were administered orally 25% (v/v) ethanol (5 g/kg body weight) once a day for 5 weeks

Values are mean±S.D. (n=6)

Means with the same letter are not significantly different ($p<0.05$)

*Significant at 5% level, **Significant at 1% level, [NS]Not significant

sustained ethanol levels in tissue. Pectin decreased protein efficiency ratio and amino acid absorption rate, so cytosolic ADH activities were decreased by 10% fiber (HF) level.

Aldehyde dehydrogenase activity

The activities of mitochondrial and cytosolic aldehyde dehydrogenase (ALDH) are shown in Table 3.

Mitochondrial ALDH activities tended to increase by chronic ethanol treatment, but cytosolic ALDH activities were decreased at 5% fiber (LF) level. The ALDH activities of 20% protein (NP) groups were higher than those of 7% protein LP) groups. Mitochondrial ALDH activities were decreased by low protein level, and this result was consistent with Wang et al. (23) who showed that treatment of mitochondria with ethanol and caused an inhibition in the import of the precursor of ALDH. Adaptive brain ALDH activation in chronic ethanol-treated groups was shown following ethanol treatment (24).

Acetaldehyde is oxidized in the brain by an ALDH which has a K_m for acetaldehyde in the micromolar range (<1 uM) (25). Thus, quite low levels of acetaldehyde will saturate this enzyme, and conceivably, this could interfere with its normal functions (26).

Cytochrome P-450 content

Table 4 shows the microsomal cytochrome P-450 content

Table 3. Effect of protein and dietary fiber levels on brain ALDH activity in rats with acute and chronic ethanol treatments

(n moles/min/mg protein)

Group[1)]	Mitochondria		Cytosol	
	Acute	Chronic	Acute	Chronic
LPNF	9.33 ± 0.89^{c}	10.03 ± 0.79^{c}	12.27 ± 1.30^{a}	8.04 ± 0.29^{e}
LPHF	10.09 ± 1.28^{c}	10.50 ± 0.76^{bc}	10.84 ± 0.76^{bc}	11.04 ± 0.84^{abc}
NPNF	9.68 ± 1.32^{c}	10.28 ± 1.02^{bc}	10.06 ± 0.83^{cd}	9.21 ± 0.33^{de}
NPHF	11.52 ± 0.81^{ab}	12.10 ± 0.76^{a}	11.06 ± 0.25^{abc}	12.01 ± 1.76^{ab}
ANOVA	F		F	
Ethanol (A)	3.67^{NS}		11.76^{**}	
Protein (B)	9.02^{**}		0.01^{NS}	
Fiber (C)	15.24^{**}		22.09^{**}	
A×B	0.00^{NS}		13.05^{**}	
A×C	0.04^{NS}		29.66^{**}	
B×C	4.14^{*}		3.81^{NS}	
A×B×C	0.03^{NS}		5.26^{*}	

[1)]See the legend of Table 1

Acute ethanol-treated group: Rats were administered orally 40% (v/v) ethanol (5 g/kg body weight) before 90 min of decapitation

Chronic ethanol-treated group: Rats were administered orally 25% (v/v) ethanol (5 g/kg body weight) once a day for 5 weeks

Values are mean±S.D. (n=6)

Means with the same letter are not significantly different ($p<0.05$)

*Significant at 5% level, **Significant at 1% level, [NS]Not significant

Table 4. Effect of dietary protein and fiber levels on brain cytochrome P-450 content in rats with acute and chronic ethanol treatments (n moles/mg protein)

Group[1)]	Cytochrome P-450	
	Acute	Chronic
LPNF	0.036 ± 0.002^{f}	0.073 ± 0.008^{bc}
LPHF	0.058 ± 0.003^{e}	0.079 ± 0.006^{abc}
NPNF	0.065 ± 0.004^{dc}	0.081 ± 0.007^{ab}
NPHF	0.072 ± 0.007^{cd}	0.084 ± 0.005^{a}

ANOVA	F
Ethanol (A)	122.85^{**}
Protein (B)	50.80^{**}
Fiber (C)	24.46^{**}
A×B	15.46^{**}
A×C	7.09^{*}
B×C	5.10^{*}
A×B×C	1.95^{NS}

[1)]See the legend of Table 1
Acute ethanol-treated group: Rats were administered orally 40% (v/v) ethanol (5 g/kg body weight) before 90 min of decapitation
Chronic ethanol-treated group: Rats were administered orally 25% (v/v) ethanol (5 g/kg body weight) once a day for 5 weeks
Values are mean±S.D. (n=6)
Means with the same letter are not significantly different ($p<0.05$)
*Significant at 5% level, **Significant at 1% level
[NS]Not significant

Table 5. Effect of dietary protein and fiber levels on brain XO activity in rats with acute and chronic ethanol treatments (uric acid n moles/mg protein/min)

Group[1)]	Xanthine oxidase	
	Acute	Chronic
LPNF	1.81 ± 0.34^{c}	2.27 ± 0.22^{bc}
LPHF	2.00 ± 0.39^{bc}	2.55 ± 0.32^{b}
NPNF	2.59 ± 0.59^{b}	3.30 ± 0.53^{a}
NPHF	3.30 ± 0.57^{a}	3.36 ± 0.58^{a}

ANOVA	F
Ethanol (A)	11.13^{**}
Protein (B)	53.53^{**}
Fiber (C)	5.28^{*}
A×B	0.24^{NS}
A×C	1.08^{NS}
B×C	0.30^{NS}
A×B×C	1.91^{NS}

[1)]See the legend of Table 1
Acute ethanol-treated group: Rats were administered orally 40% (v/v) ethanol (5 g/kg body weight) before 90 min of decapitation
Chronic ethanol-treated group: Rats were administered orally 25% (v/v) ethanol (5 g/kg body weight) once a day for 5 weeks
Values are mean±S.D. (n=6)
Means with the same letter are not significantly different ($p<0.05$)
*Significant at 5% level, **Significant at 1% level
[NS]Not significant

of rat brain.

Chronic ethanol treatment significantly the increased level of P-450 in brain, especially in LP groups and the effect of protein was remarkable in the acute ethanol-treated groups. Protein deficiency decreased clearance rate of ethanol, and reduced P-450 content (21). But the effect of dietary fiber on cytochrome P-450 contents was not seen except in the LP groups of acute ethanol treatment.

Cohn et al. (27) and Sasame et al. (28) identified cytochrome P-450 in the brain. P-450 plays a key role in metabolizing a variety of endogenous as well as exogenous compounds. In addition to P-450, the other enzymatic component of the mixed function oxidase (MFO) system is NADPH-cytochrome P-450 reductase. Although the liver is a major site for P-450 mediated metabolism, other organs, such as the colon, lung as well as the brain also exhibit MFO activity (29).

Xanthine oxidase activity

The effect of protein and dietary fiber levels on the activity of xanthine oxidase(XO) in cytosol was shown in Table 5.

The brain activities of XO tended to increase in chronic ethanol-treated groups, especially in the NPNF group. Oei et al. (30) proposed that the formation of superoxide free radicals during acetaldehyde metabolism by XO contributes to ethanol-induced pathology. Thus, free radical generation was aggravated by chronic ethanol treatment. XO activities in 7% protein (LP) diet groups were siginificantly decreased compared with 20% protein (NP) diet both in the acute and chronic ethanol-treated rats. Wainio et al. (31) have also reported a siginificant decrease in the XO activity by a low protein diet. Therefore, these results indicate that the enzyme response was very sensitive to changes in dietary protein level. Both in low and normal protein groups, addition of 5% pectin (NF) to the diet reduced the activity of XO, although not significantly.

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