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Effects of Alanine and Glutamine Supplementation on Alcohol Metabolism in ICR Mice

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ABSTRACT

This study was conducted to investigate the effects of oral supplementation of alanine and glutamine on alcohol metabolism. The subjects were 70 male ICR mice weighing 25 - 30 g. The animals were raised on standard rations after weaning. After 24 hours of fasting, all the animals were given a peritoneal injection of 20% alcohol. Then, they were randomly divided into two groups: control and experimental. Fifteen minutes after the injection of alcohol, the mice in the experimental group were given an oral solution of alanine (5 mM, 2 g/kg B. W.) and glutamine (5 mM, 2 g/kg B.W.). The concentration of alcohol in the blood was measured in all the mice 20 minutes after they received the alcohol, and the measurements continued every 20 minutes up to 140 minutes. The experimental group sustained lower blood alcohol levels at every 20 minute time interval compared to the control group, showing that oral supplementation of alanine and glutamine increases the rate of alcohol metabolism. Furthermore, the total amount of alcohol remaining in the blood, determined by using the Area Under the Curve (AUC) method, was lower in the group supplemented with alanine and glutamine, However, the effectiveness of alanine and glutamine in increasing the rate of alcohol metabolism, compared to the control group, diminished with time throughout the experiment. In conclusoin, alanine and glutamine supplementation appears to promote alcohol metabolism shortly after alcohol intake.

KEY WORDS: alcohol, alanine, glutamine.

INTRODUCTION

Alcohol has been shown to have potentially harmful effects in humans. Alcohol directly interferes with brain functions by suppressing the central nervous system. Alcohol is an important factor in developing fatty livers, by increasing fat accumulation in hepatic cells through reducing the oxidation of fatty acids.[1][2] Alcohol also induces hepatitis due to inflammation and necrosis of hepatic tissue, and cirrhosis due to accumulation of collagen.[3] When alcohol and drugs are metabolized by microsomal enzymes, excessive releases of free radicals occur as intermediate metabolites.[4] Alcohol stimulates the secretion of gastric acid when it is taken with aspirin or other drugs, and results in gastric inflammation, gastric ulcers and severe bleeding.[5][6]

Alcohol is referred to as an "empty calorie" substance, as it contains no nutrients other than calories. Alcohol also interferes with the absorption of micronutrients such as vitamins and minerals, leading to the loss of functions in many parts of body.

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Alcohol is easily absorbed in the small intestine, and 90% of it is metabolized in the liver.[8] There are three pathways for alcohol metabolism in the body: 1) Alcohol Dehydrogenase (ADH) which utilizes the coenzyme NAD^+ in the cytosol,[9] 2) the Microsomal Ethanol Oxidizing System (MEOS) which utilizes NADPH and Cytochrome P 450 in microsomes with chronic or high alcohol intake,[10][11] and 3) the catalase-hydrogen peroxide system in the peroxisome.[12] The most important, rate-limiting, step in alcohol metabolism is the conversion of alcohol to acetaldehyde by ADH using coenzyme NAD^+. One mole of NAD^+ is reduced to NADH when alcohol is oxidized to acetaldehyde by ADH.[13] ADH combines with NAD^+, and the activated ADH-NAD+ complex combines with alcohol, which is then oxidized to acetaldehyde. After oxidation, the ADH-NADH complex is separated, and this process of separation is the rate-limiting step in alcohol metabolism. In hepatic cells, the rate of separation of the ADH-NADH complex from the acetaldehyde is determined by the amount of NADH. Thus, alcohol metabolism is determined by the re-oxidation of NADH,[14] that is, the regeneration of NAD^+.[15]

In order to accelerate alcohol metabolism, many me-

thods have been proposed to facilitate the regeneration of NAD^+. One example of these methods is use of the enzyme quinone reductase (QR). QR is considered to promote the rate of alcohol metabolism by reducing quinone to hydroquinone through oxidizing NADH to NAD^+. The supplementation of quinone substrates (tert-butyl quinone, menadione, menadione sodium sulfate), and the dietary intake of butylated hydroxyanisole (BHA) which can induce QR, were reported to have increased alcohol metabolism.[16] Also, the conversion of pyruvate to lactate is a reduction process, which is coupled with alcohol oxidation[17]; pyruvate is reduced to lactate with the help of the coenzyme NADH. The supply of large amounts of fructose, which is converted to pyruvate is also reported to have increased alcohol metabolism by 25–75%.[18][19] Another study reported that alanine, which is converted to pyruvate by the transamination process, can increase alcohol metabolism, and this effect is due to increased use of NADH in the cytosol.[20][21]

This study was carried out to develop methods for promoting alcohol metabolism by using alanine and glutamine. Alanine can be directly converted to pyruvate, and glutamine is thought to help with the supply of pyruvate indirectly and to activate the urea cycle by the production of NH_3.

MATERIALS AND METHODS

1. Experimental animals and diet

Seventy male ICR mice were raised in the animal laboratory of the Medical College of Inha University to become 25–30 g body weight. The animal laboratory had a 12-hour light/dark cycle, and the temperature was maintained at 20–22℃. Animals were given standard rations (Sam-Yang) and water, *ad libitum*.

2. Alcohol injection and blood collection

All animals were fasted for 24 hours prior to the experiment for glycogen depletion and then weighed. At the beginning of the experiment, all mice were injected intraperitoneally with 20% alcohol diluted in saline solution at a dosage of 2 g/kg body weight. The animals were randomly divided into two groups: control (alcohol only), and experimental (alcohol + alanine · glutamine). Fifteen minutes after the alcohol injection, each member of the experimental group was orally given 5 mM alanine and 5 mM glutamine at a dosage of 2 g/kg body weight. The dosages of alanine and glutamine were taken according to the result of our preliminary study. Then 0.5 ml blood was taken by heart puncture from 5 animals from each of the control and experimental groups every 20 minutes, up to 140 minutes. This sequence is illustrated in Fig. 1. No anaesthetics were used in order to avoid their possible influence on alcohol metabolism.

3. Determination of blood alcohol

Each 0.5 ml blood sample was added to a tube which contained 4.5 ml of 10% TCA, and was centrifuged at 3000 × g for 5 minutes. The supernatant was taken to measure alcohol concentration using a spectrophotometer. The method employed was of Buchner and Redetzki which uses the ADH enzyme.[22]

4. Data analysis

The rate of alcohol metabolism was expressed as blood alcohol concentration over time using the Havard Graph program, and the remaining blood alcohol from the time of alcohol injection up to 140 minutes was calculated by using the Area Under the Curve (AUC), which is the Widmark Method.[23] Data were statistically analyzed by calculating means and standard deviations; the difference between the control and experimental groups was tested by the Student's t-test with a statistical significance level of $p < 0.05$.

RESULTS

1. Change of blood alcohol concentration with time

Fig. 2 shows average blood alcohol concentrations for

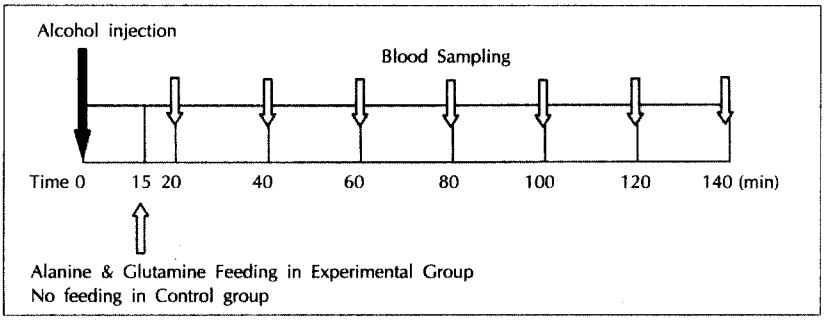

Fig. 1. Experimental design.

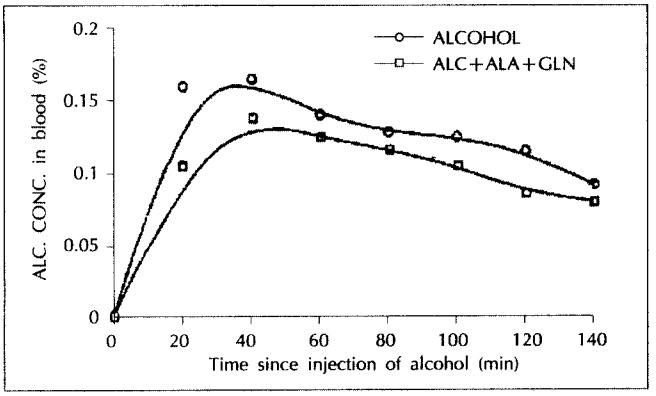

Fig. 2. Effects of alanine and glutamine supplementation on blood alcohol concentration in ICR mice. *: Each data point represents the average blood alcohol concentration of 5 subjects.

each of the experimental and control groups, at intervals of 20 minutes beginning 20 minutes from the time of alcohol injection and continuing until 140 minutes after alcohol injection. Compared to the control group, the experimental group sustained lower blood alcohol levels throughout the trial. Although there were no significance, the differences of the blood alcohol concentration in two groups were the highest between 20 minutes and 40 minutes. However, the differences became small for the remainder of the trial.

2. Comparison of remaining blood alcohol concentration

Table 1 compares the remaining blood alcohol concentrations between the control and experimental groups, as measured by the Area Under the Curve method over the 140 minutes experimental period. The experimental group supplemented with alanine and glutamine had lower remaining blood alcohol concentration compared to the control group which had no supplementation ($p < 0.05$).

DISCUSSION AND CONCLUSION

Most absorbed alcohol is metabolized by Alcohol Dehydrogenase (ADH) in the cytosol of the liver. ADH requires the coenzyme NAD^+ and the rate-limiting step in this process is regeneration of NAD^+ from NADH, which is coupled with the reduction of pyruvate to lactate. Thus, increased supplies of pyruvate would promote alcohol metabolism.

Alanine was used in this trial as a method of supplying pyruvate, because alanine is converted to pyruvate by the transamination in the liver and at the same time provides NH_3 to alpha-ketoglutarate for the production of glutamate. The pyruvate from alanine would be converted to lactate, and would regenerate NAD^+ which is essential for the ADH reaction, thus promoting alcohol metabolism. Furthermore, glutamine supplementation would accelerate the coupling reaction of alcohol-acetaldehyde and pyruvate-lactate by eliminating NH_3, thereby activating urea cycle.[24] Glutamine is converted to glutamate by deamination, and glutamate, in turn, will be converted to alpha-ketoglutarate. Therefore, glutamine will promote the transamination of alanine into pyruvate, and the NH_3 produced will activate the urea cycle.

Table 1. AUC of blood alcohol concentration during the 140 minute period after alcohol injection

Group	Area under the curve (AUC)
Control (alcohol only)	17.254 ± 0.062
Experimental (alcohol + Ala · Gln)	14.205 ± 0.073*

Values are expressed as mean ± S.D
*: significantly different from control ($p < 0.05$)
Ala: alanine
Gln: glutamine

The simultaneous supplementation of alanine and glutamine would promote alcohol metabolism as well as activation of the urea cycle, by ensuring increased supplies of NAD^+ in hepatic cells, hence promoting alcohol metabolism and reducing toxicity in the liver.

The results of this experiment show that alcohol metabolism is promoted by alanine and glutamine supplementation. It is thought that alanine improves alcohol metabolism through increased supplies of pyruvate, and that glutamine accelerates alcohol metabolism by activating the urea cycle. However, the promoting effect of alanine and glutamine on alcohol metabolism in this experiment became more limited over time.

In addition to alanine, the intake of glucose/fructose and quinone - which regenerates NAD^+ by being reduced to hydroquinone through the reaction of qinone reductase (QR) - would promote alcohol metabolism considerably. The development of drugs which would promote alcohol metabolism would minimize alcohol toxicity and will be useful in treatment of alcoholics or of the diseases induced by alcohol.

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