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Abstract

Membrane fluidity in human erythrocytes was measured by a spin label method using an electron spin resonance spectrometer in healthy volunteers after ingestion of alcohol (1.5 ml of whisky/kg body weight). Fluidity in the lipid bilayer closer to the hydrophilic face decreased at 30 min and 90 min, and fluidity in the hydrophobic core decreased at 90 min after ingestion of alcohol. In the same experiment, the level of thiobarbituric acid reactive substances in the serum decreased 30 min after ingestion of alcohol, and the triglyceride level increased and free fatty acid level decreased, and serum superoxide dismutase activity increased 150 min after ingestion. Furthermore, membrane fluidity in human erythrocytes was examined in patients with alcohol dependence syndrome who had not any alcohol for about 26 months. Erythrocyte membrane fluidity of patients with alcohol dependence syndrome was not different from that of healthy controls. However, erythrocyte membrane fluidity of the lipid bilayer closer to the hydrophilic face increased in patients who had concomitant liver cirrhosis compared with those who did not. These results suggest that alcohol affects temporal change of membrane fluidity in human erythrocytes.

KEYWORDS: erythrocyte membrane fluidity, alcohol, superoxide dismutase activity, lipid peroxide, alcohol dependence syndrome

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Membrane fluidity in human erythrocytes was measured by a spin label method using an electron spin resonance spectrometer in healthy volunteers after ingestion of alcohol (1.5 ml of whisky/kg body weight). Fluidity in the lipid bilayer closer to the hydrophilic face decreased at 30 min and 90 min, and fluidity in the hydrophobic core decreased at 90 min after ingestion of alcohol. In the same experiment, the level of thiobarbituric acid reactive substances in the serum decreased 30 min after ingestion of alcohol, and the triglyceride level increased and free fatty acid level decreased, and serum superoxide dismutase activity increased 150 min after ingestion. Furthermore, membrane fluidity in human erythrocytes was examined in patients with alcohol dependence syndrome who had not any alcohol for about 26 months. Erythrocyte membrane fluidity of patients with alcohol dependence syndrome was not different from that of healthy controls. However, erythrocyte membrane fluidity of the lipid bilayer closer to the hydrophilic face increased in patients who had concomitant liver cirrhosis compared with those who did not. These results suggest that alcohol affects temporal change of membrane fluidity in human erythrocytes.

Key words: erythrocyte membrane fluidity, alcohol, superoxide dismutase activity, lipid peroxide, alcohol dependence syndrome

Erythrocyte membrane fluidity has been reported to be related with the ratio of cholesterol to phospholipids in the red cell membrane (1, 2) and with the level of lipid peroxide in rat synaptosomes (3). Superoxide and hydroxyl radicals in synaptosomes of the rat have also been reported to affect erythrocyte membrane fluidity (4).

Abnormalities in the shape of red cells have also been reported in alcoholics (5). Halsall reported macrocytosis and an increase in the ratio of free cholesterol to phospholipids in erythrocytes after ingestion of excessive amounts of alcohol, and suggested this to be the direct effect of alcohol on erythrocyte membranes (6).

In the present paper, first, membrane fluidity of erythrocytes was examined by spin label method using an electron spin resonance spectrometer in healthy men after ingestion of alcohol to clarify the effect of alcohol on membrane fluidity. Then, membrane fluidity of erythrocytes in patients with alcohol dependency syndrome (ADS) was compared with that in healthy men after acute alcohol ingestion.

Alcohol is metabolized in the liver, and abnormal lipid metabolism, such as the accumulation of triglyceride, has been reported in the liver of alcoholics (5). Therefore, the effect of alcohol on the serum levels of triglyceride, free fatty acid, thiobarbituric acid reactive substances (TBARS) and superoxide dismutase (SOD) activity was examined in serum of the healthy men.

Subjects and Methods

Subjects. Eight healthy men 20–22 years of age (average 21 years) were given 1.5 ml/kg body weight of whisky (pure alcohol 0.5 g/kg body weight), and blood samples were collected at 30, 90 and 150 min after ingestion.

Fifty-five patients (52 men and 3 women) with ADS, 30–82 years of age (average 50.8 years) were evaluated to study the long-term effects of alcohol. They used to

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consume alcohol in the range of 100–150 ml (calculated as pure alcohol) every day, but they had not taken alcohol for an average of 26 months (range 1–115 months) at the time of enrollment. There were four cases of liver cirrhosis and 10 cases of diabetes mellitus among the patients with ADS. The control group for ADS was composed of 35 healthy volunteers (26 men and 9 women), 20–69 years of age (average 44.9 years) who had no history of alcohol dependence and they had not taken alcohol at least 24 h before the experiment.

All volunteers and patients were informed of the nature of this study and concented to participate.

Chemicals. Spin labels 5- and 16-doxyl stearic acid (5-, 16-DS) were purchased from SIGMA Chemical Co. (St. Louis, MO, USA) and used to measure the order and motion parameters, respectively. All other chemicals and reagents were of the highest grade available.

Preparation of erythrocytes. Erythrocytes were prepared from whole blood by centrifugation at 3,000 rpm for 15 min. The erythrocyte pellet was then washed twice with two volumes of physiological saline at 3,000 rpm for 10 min. Erythrocyte pellets were rinsed again with physiological saline in the same way. Final pellets were resuspended with two volumes of saline for use in the membrane fluidity assay.

Membrane fluidity analysis. Eight micrograms of spin labels 5-DS and 16-DS per mg protein were dissolved in ethanol then evaporated under nitrogen. One hundred microliters of erythrocyte suspension was added to it and then sample was mixed for 2 min by a vortex mixer. Sample was then placed in a capillary tube and the spin labels were assayed using an electron spin resonance (ESR) spectrometer (JEOL JES-FE-1XG, Tokyo). ESR spectrometry conditions were as follows: magnetic field, 322 ± 5 mT and 10 mT; modulation, 0.2 mT; response, 0.3 sec; amplitude, $3.2 \times 1{,}000$; sweep time, 2 and 4 min; and temperature, 37℃. The polarity-correlation order parameter was calculated from the spectra according to the method of Hubbel and McConnell (7) in the 5-DS-labeled samples. The motion parameter, corresponding to the rotational correlation time in the hydrophobic region, was used for the 16-DS-labeled samples, as explained by Eletr and Inesi (8).

Superoxide dismutase (SOD) assay. SOD activity in serum and erythrocytes was assayed using spin trap, 5,5-dimethyl-1-pyrroline-1-oxide by an ESR spectrometer (9).

Assay of thiobarbituric acid reactive substances (TBARS). TBARS in serum were analyzed using thiobarbituric acid (10).

Assay of alcohol and acetaldehyde. Alcohol in serum was analyzed using a BMY kit (Boehringer-Mannheim, Germany), and acetaldehyde in serum was analyzed by head space gas chromatography (11).

Assay of triglyceride and free fatty acid. Serum levels of triglyceride and free fatty acid were analyzed using a Diatest TG kit (Daiichi Pure Chemical Co., Ltd., Tokyo) and Nescauto Nefa-Vz kit (Nippon Shoji Kaisha Ltd., Osaka), respectively.

Protein assay. Protein was assayed using a protein assay reagent (PIRCE Rockford, IL, USA).

Statistical analysis. Groups of samples were compared by unpaired Student's *t*-test.

Results

In the case of acute ingestion of alcohol in healthy young men, the order parameter of 5-DS in erythrocytes increased significantly 30 min after ingestion of alcohol and was still high 90 min after ingestion in comparison with 0 time, indicating that fluidity of the lipid bilayer closer to the hydrophilic face of the membrane was significantly decreased. The increased parameter returned to normal 150 min after the ingestion of alcohol. The motion parameter of 16-DS in erythrocytes significantly increased 90 min after the ingestion of alcohol, indicating a significant decrease in membrane fluidity in the core region of the lipid bilayer. It returned to normal 150 min after ingestion (Fig. 1). The triglyceride level significantly increased and free fatty acid level significantly decreased in the serum of healthy men 150 min after ingestion of alcohol (Fig. 2). Serum TBARS of healthy subjects was significantly decreased 30 min after ingestion and the level returned to normal after 90 min. While SOD activity increased significantly 150 min after ingestion in the serum of healthy subjects, the change in the erythrocytes was not significant at any time after the ingestion (Fig. 3). Levels of acetaldehyde and ethanol increased sharply up to 30 min after the ingestion of alcohol, and both levels gradually decreased thereafter although they were still high at 150 min (Fig. 4). No relationship between membrane fluidity (either in the hydrophobic core region or the lipid bilayer closer to the hydrophilic face of the membrane), and serum alcohol or acetaldehyde of healthy subjects after ingestion of alcohol was found (Fig. 5).

In the case of patients with ADS, there was no

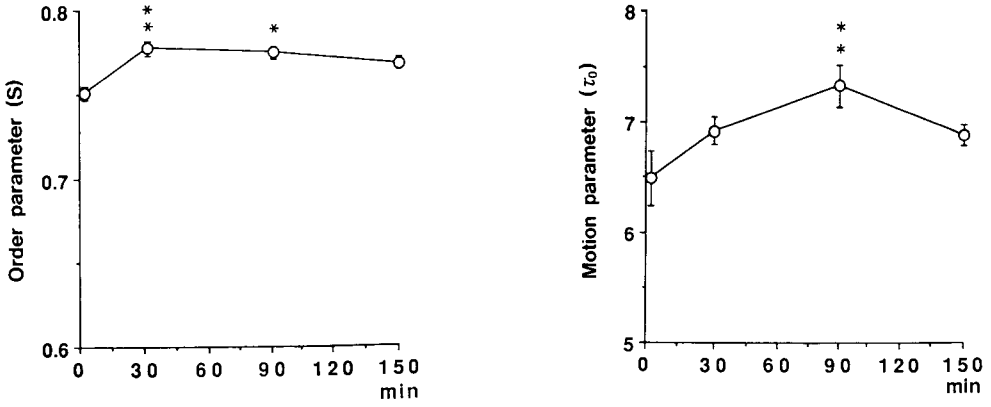

Fig. 1 Effects of alcohol on human erythrocyte membrane fluidity. Each value represents the mean ± SEM of 8 subjects. $*P < 0.05$, $**P < 0.01$ *vs* 0 min.

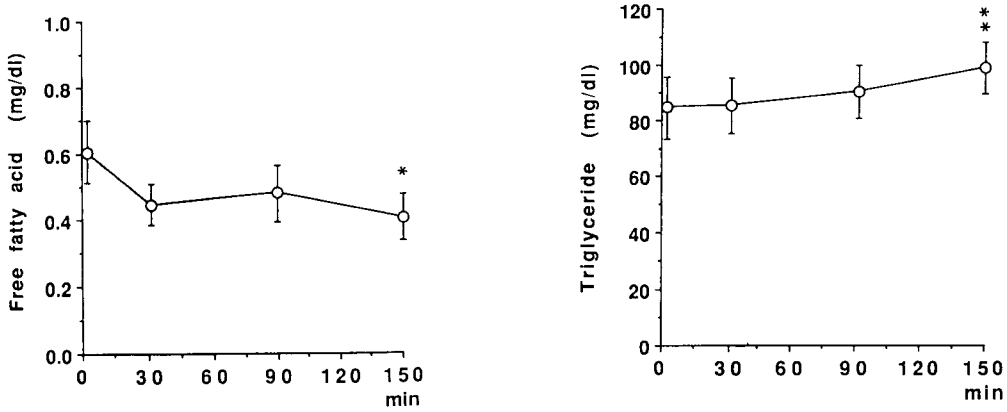

Fig. 2 Effects of alcohol on levels of triglyceride and free fatty acid in human serum. Each value represents the mean ± SEM of 8 subjects. $*P < 0.05$, $**P < 0.01$ *vs* 0 min.

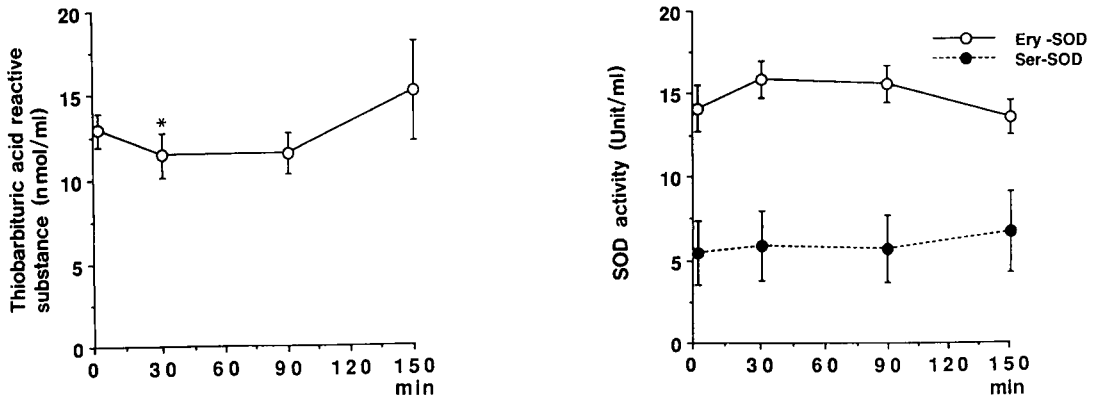

Fig. 3 Effects of alcohol on thiobarbituric acid reactive substances (TBARS) and superoxide dismutase (SOD) activity in human serum and erythrocyte. Each value represents the mean ± SEM of 8 subjects. $*P < 0.05$ *vs* 0 min. Ery, erythrocyte; Ser, serum.

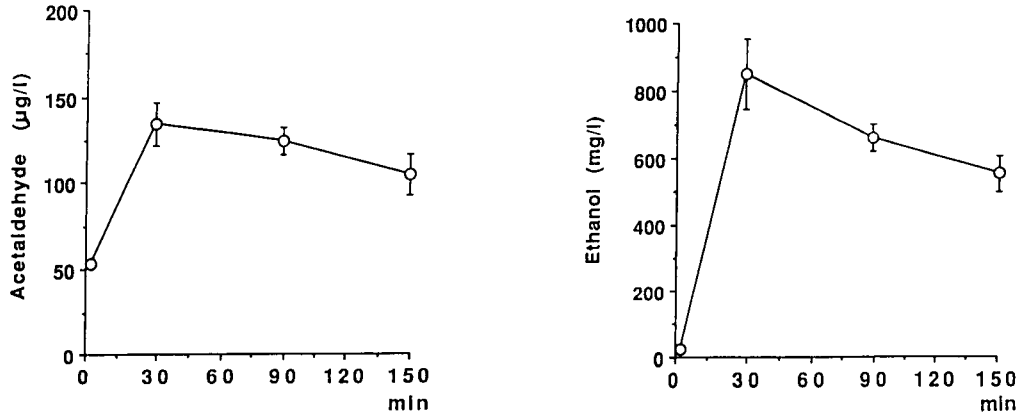

Fig. 4 Acetaldehyde and ethanol levels in human serum after acute ingestion of alcohol. Each value represents the mean ± SEM of 8 subjects.

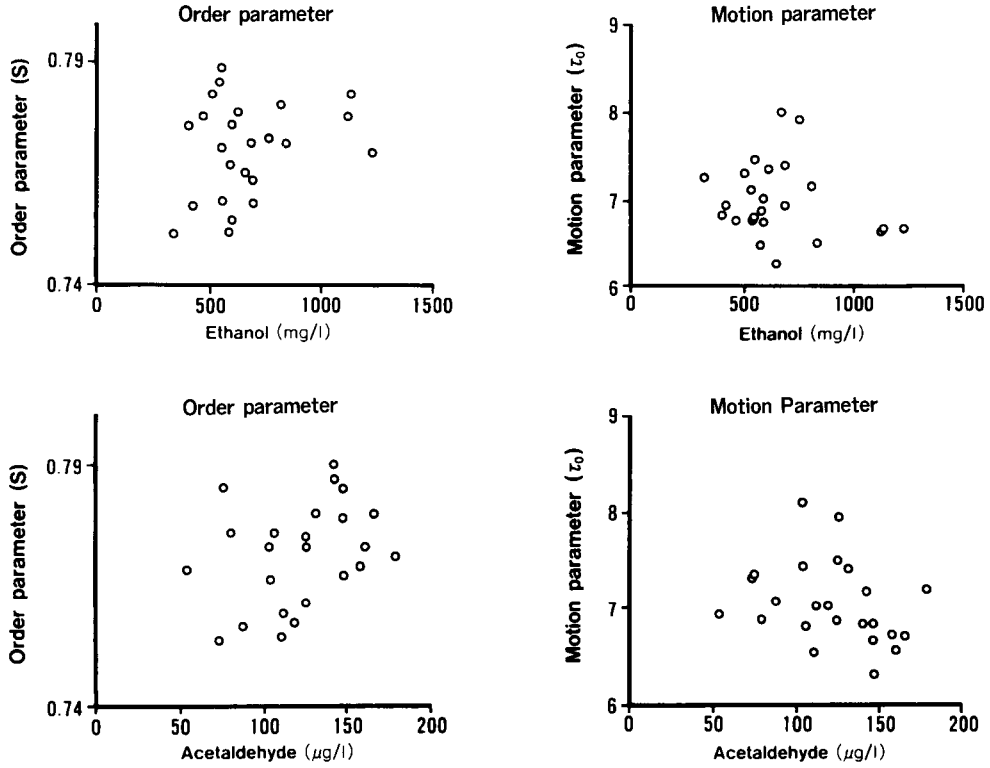

Fig. 5 Relationship between membrane fluidity in erythrocyte and ethanol level or acetaldehde level in serum of healthy subjects after acute ingestion of alcohol.

significant difference in membrane fluidity (either in the hydrophobic core region or the lipid bilayer closer to the hydrophilic face of the membrane) compared to controls. Among the patients, the order parameter in erythrocytes of patients with ADS and liver cirrhosis was significantly lower than that of patients with ADS without cirrhosis. In the patients with ADS, there was no difference in the order and motion parameters in the erythrocytes of

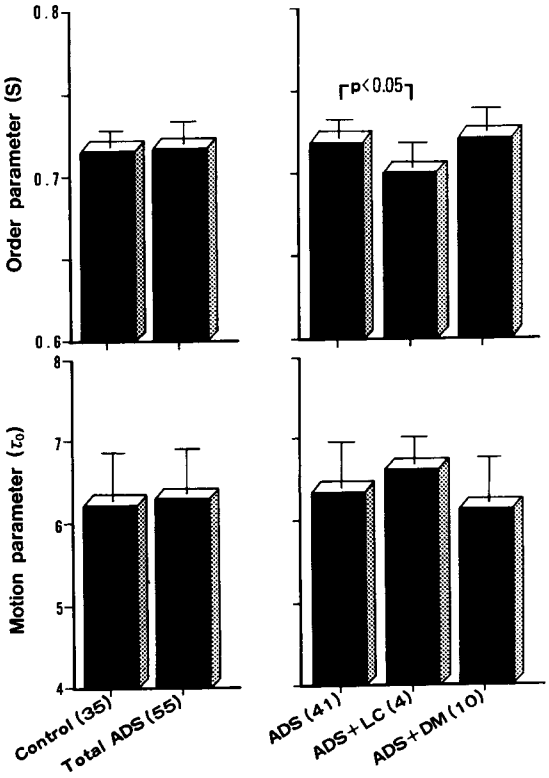

Fig. 6 Erythrocyte membrance fluidity in patients with alcohol dependence syndrome (ADS), with concomitant diabetes mellitus (DM) or liver cirrhosis (LC). Each bar represents the mean ± SD.

patients who had diabetes mellitus compared to those who did not (Fig. 6).

Discussion

The present study showed that acute alcohol ingestion affected the erythrocyte membrane fluidity of human subjects. Chronic alcohol administration is known to change the composition of lipids in the human red cell membrane (1, 6, 12), and also to change the cell membrane fluidity (13). In our experiment, acute alcohol ingestion temporarily decreased erythrocyte membrane fluidity in both the lipid layer closer to the hydrophilic face and in the hydrophobic core region. These decreases of membrane fluidity may be induced by transitory changes in the molecular conformation of phospholipids, though this will be the subject of further study.

Lieber *et al.* (14) reported that serum triglycerides in humans increased after ingestion of alcohol. Our results also showed the increase of triglyceride level in the serum of healthy subjects 150 min after ingestion. SOD activity in serum increased in healthy subjects 150 min after ingestion (Fig. 3). In the metabolism of alcohol, it has been suggested that free radicals are generated from acetaldehyde via xanthine oxidase (15, 16). Furthermore, hydroxyl radical and superoxide have been reported to decrease neuronal membrane fluidity (4). Similarly, the decrease of erythrocyte membrane fluidity in human subjects after the ingestion of alcohol is thought to be due to the generation of free radicals in the metabolism of alcohol. The increase of SOD activity in serum 150 min after the ingestion of alcohol might be induced to scavenge superoxide generated in the metabolism of alcohol.

Lipids in erythrocytes are affected by serum lipids (12). The decrease of TBARS in serum of human subjects 30 min after the ingestion of alcohol might be due to degradation of lipid in the liver by alcohol. No significant correlation was found between fluidity in the hydrophobic regions and lipid bilayer closer to the hydrophilic face of erythrocyte membranes and serum alcohol or acetaldehyde. Kawai *et al.* (17) have shown that acute ethanol ingestion (one ingestion of 5 g/kg ethanol) into rats increased membrane fluidity and decreased the ratio of sphingomyeline to phosphatidylcholine in the liver plasma membrane transiently 3–6 h after ingestion. The results in rats and in the present study differ, and this discrepancy seems to be due to the different composition in membranes in rats and humans (18).

There was no change of fluidity in the hydrophobic regions or the lipid bilayer closer to the hydrophilic face of erythrocyte membranes in patients with ADS, who had not consumed alcohol for between 4 and 8 days, and whose daily average alcohol consumption from self-reported drinking history was estimated at 60–374 g of ethanol per day for the preceding 2 weeks (19). As our patients with ADS have abstained from taking alcohol for 26 months, alcohol-induced changes in membrane fluidity returned to normal. The order parameter of erythrocytes of patients with ADS with liver cirrhosis significantly decreased compared to those without cirrhosis. There was no significant change in the order parameter in erythrocytes of patients with ADS and diabetes mellitus. Cooper (13) reported that the ratio of free cholesterol to phospholipids in erythrocyte membranes increased in patients with liver cirrhosis. The change in membrane

fluidity has been suggested to be related to the lipid composition, unsaturated fatty acid level, and cholesterol level (3). Therefore, the increased fluidity in erythrocyte membranes in the lipid bilayer closer to the hydrophilic face observed in the present study may be due to these abnormalities in the lipid composition of erythrocyte membranes in patients with ADS with concomitant liver cirrhosis.

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