J. Food Sci. Nutr.
Vol. 3, No. 1, p. 56~61 (1998)

Effect of Dietary Vitamin A on Plasma Membrane Property and Ultrastructure in Ethanol-administered Rat Liver

Kyung-Mi Yang, Jung-Sook Seo† and Hyun-Gug Cho*

Dept. of Food and Nutrition, and
**Dept. of Biology, Yeungnam University, Kyongsan 712-749, Korea*

Abstract

This study was conducted to investigate the effect of dietary supplementation of vitamin A on the membrane property and ultrastructure in ethanol-administered rat livers. Male Sprague-Dawley rats weighing of 130~150g were fed with experimental diets for 7 weeks. The diets contained different types of vitamin A which were β-carotene, retinyl acetate and retinoic acid. After feeding the experimental diets for 7 weeks, a dose of 3.0g ethanol(30%, W/V)/kg B.W was injected to rats intraperitoneally. Control rats received 0.9% saline containing isocaloric sucrose instead of ethanol. Plasma membrane fluidity of liver decreased in rats fed with vitamin A-deficient diet with ethanol as compared to that of control rats. Fluidity change of liver plasma membrane that ethanol had induced was influenced by dietary supplementation of vitamin A, but not influenced by the type of supplemented vitamin A. The ultrastructural changes of hepatic mitochondria were observed in some rats such as vitamin A-deficient rats with ethanol. Inadequate consumption of vitamin A contributed to ultrastructural changes such as swelled mitochondria occurred by ethanol-induced hepatotoxicity. Although accurate mechanism involved in the plasma membrane-stabilizing effect of vitamin A is still unclear, dietary supplementation of vitamin A such as retinyl acetate is needed to modulate this change. The direct involvement of membrane property on the cell damage caused by ethanol treatment remains to be established.

Key words: ethanol, vitamin A, membrane fluidity, ultrastructure

INTRODUCTION

Alcohol-induced tissue damage results from some direct toxic effects, which have now been linked to the metabolism of ethanol(1). Acetaldehyde is a major metabolic product of ethanol and is found in high concentrations in serum during alcohol abuse. Acetaldehyde, however, has been shown to bind to hepatocyte membranes *via* intermediary Schiff's base formation. The adduction of acealdehyde to plasma membranes of liver cell may have an effect on membrane structure(2).

Chronic ethanol consumption enhances microsomal degradation of retinoids and then promotes their depletion and associated pathology(1). As the interface between plasma and the cytoplasm, plasma membranes are a critical part of the transport system for retinol, but the mechanisms by which the vitamin is transferred from extracellular retinol binding protein to the intracellular binding proteins have not yet been clearly established(3).

Several studies have also correlated the membrane effects of retinoids and their biological activity(4,5). Wassall et al.(5) have shown that retinoids modify the properties of membranes. All-*trans*-retinoic acid markedly enhances permeability, while all-*trans*-retinol or retinal produces slight increases.

Ethanol is one of the important factors influencing membrane fluidity. The change in fluidity may result from a disturbance of the dynamic equilibrium of membrane phospholipids. Many membrane-bound enzymes are affected by their fluid environment and membrane transport activity(6).

Damage to the plasma membranes is commonly associated with liver injury and vitamin A is well known to affect the stability of biological membranes(7). Carotenoids are also postulated to play a physiological role in modifying structural and dynamic properties of biomembranes and model membranes(8). The membrane-fluidizing effect of β-carotene may be also important for its antioxidant activity, which is believed to be a basis of its anti-disease properties(9,10).

†Corresponding author

Allopurinol concentration achieved in cerebellum following a regimen treatment of 146μmol/kg is not clearly defined. Nevertheless some reports showed that following allopurinol administration to animals the allopurinol concentration in cortical perfusates(25) or in cerebrospinal fluid(26) was about $2\sim3\times10^{-5}$ mol l^{-1}.

The vitamin E content in the central nervous system was itself about 5×10^{-5}mol l^{-1}(27). Therefore, the interaction of TH+All was observed at those concentrations in the present homogeneous system. As can be seen in Fig. 4, the result obtained in this case is in very good agreement with all the other obtained yields G(-TH). It can therefore be concluded that the biological TH/All concentration ratio seems to be, at least in homogeneous solution, in favor of the proposed mechanism of allopurinol action which might be relevant to *in vivo* situations.

REFERENCES

1. Nordmann, R. : Alcohol and antioxidant systems. *Alcohol.*, **29**, 513(1994)
2. Renis, M., Calabress, V., Russo, A., Calderone, A., Barcellona, M. L. and Rizza, V. : Nuclear DNA strand breaks during ethanol-induced oxidative stress in rat brain. *FEBS Lett.*, **390**, 153(1996)
3. Bondy, S. C. and Guo, S. X. : Effect of an NMDA receptor antagonist and ganglioside GMI derivative upon ethanol-induced modification of parameters of oxidative stress in cerebral brain regions. *Brain Res.*, **716**, 165(1996)
4. Rouach, H., Ribiere, C., Park, M. K., Saffar, C. and Nordmann, R. : Lipid peroxidation and brain mitochondrial damage induced by ethanol. *Bioelectrochem. Bioenerg.*, **18**, 211 (1987)
5. Emmerson, B. T. : Clinical pharmacology of drugs used in hyperuricemia. In "*Hyperuricemia and gout in clinical practice*" Emmerson, B. T.(ed), AIDS Health Science Press, p.123(1983)
6. Palmer, C., Vannuci, R. C. and Towfighi, J. : Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. *Pedriatrc Res.*, **27**, 332(1990)
7. Mun, K. C., Kwak, C. S. and Kwon, K. Y. J. : The protective effect of allopurinol on cholestatic liver injury induced by bile ligation. *Korean Med. Sci.*, **11**, 239(1996)
8. Parks, D. A. and Granger, D. N. : Xanthine oxidase: biochemistry, distribution and physiology. *Acta Physiol. Scand.*, **548**, 87(1986)
9. Das, D. K., Engelman, R. M., Clement, R., Otani, H., Prasad, M. R. and Rad, P. S. : Role of xanthine oxidase inhibitor as free radical scavenger: a novel mechanism of action of allopurinol and oxypurinol in myocardial salvage. *Biochem. Biophys. Res. Commun.*, **148**, 314(1987)
10. Cockerham, K. G., Arroyo, C. M. and Hampton, J. D. : Effect of 4-hydroxypyrazol(3,4-d)pyrimidine(allopurinol) on post irradiation cerebral blood flow: implications of free radical involvment. *Free Radical Biol. Med.*, **4**, 279(1988)
11. Moorhouse, P. C., Grootveld, M., Halliwell B., Quinlan, J. G. and Gutteridge, J. M. C. : Allopurinol and oxypurinol are hydroxyl radical scavengers. *FEBS Lett.*, **213**, 23 (1987)
12. Augustin, A. J., Boer, T., Blumener, S. H., Lutz, J. and Spiltznas, M. : Free radical scavenging and antioxidant activity of allopurinol and oxypurinol in experimental lens-induced uveitis. *Invest. Ophthalmol. Vis. Sci.*, **35**, 3897(1994)
13. Peterson, D. A., Kelly, B. and Gerrard, J. M. : Allopurinol can act as an electron transfer agent. Is this relevant during reperfusion injury? *Biochem. Biophys. Res. Commun.*, **137**, 76(1986)
14. Liebler, D. C. and Burr, J. A. : Antioxidant stoichiometry and the oxidative fate of vitamin E in peroxyl radical scavenging reaction. *Lipid*, **30**, 789(1995)
15. Tappel, A. L. : Vitamin E and free radical peroxidation of lipid. *Ann. N.Y. Acad. Sci.*, **203**, 12(1972)
16. Chan, A. C. : Parteners in defense, vitamin E and vitamin C. *Can. J. Physiol. Pharmacol.*, **71**, 725(1993)
17. Jore, D. and Ferradini, C. : Radiolytic study of α-tocopherol oxidation in ethanolic solution. *FEBS Lett.*, **183**, 299(1985)
18. Jore, D., Kaouadji, N. and Ferradini, C. : Vitamin E and correlated antioxidants: a gamma radiolysis study. *Adv. Exp. Med. Biol.*, **264**, 151(1990)
19. Mihara, M., Uchiyama, M. and Fukuzawa, K. : Thiobarbituric acid value on fresh homogenate of rat as a parameter of lipid peroxidation in aging, CCl_4 intoxication and vitamin E deficiency. *Biochem. Med.*, **23**, 302(1980)
20. Vatassery, G. T. and Hagen, D. F. : A liquid chromatographic method for quantitative determination of α -tocopherol in rat brain. *Anal. Biochem.*, **79**, 129(1977)
21. Cooper, A. J. L., Pulsinelli, W. A. and Duffy, T. E. : Glutathion and ascorbate during ischemia and postischemic reperfusion in rat brain. *J. Neurochem.*, **35**, 1242(1980)
22. Lowry, O. H., Rosebrough, M. J., Farr, A. L. and Randall, R. J. : Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265(1951)
23. Jore, D., Champion, B., Kaouadji, N., Jay-Gerin, J. P. and Ferradini, C. : Radiolysis of ethanol and ethanol-water solvents : a tool for studying bioradical reactions. *Radiat. Phys. Chem.*, **32**, 443(1988)
24. Sarneto, A., Linder, N. and Raivio, K. O. : Organ distribution and molecular forms of human xanthine dehydrogenase/xanthine oxidase protein. *Lab. Invest.*, **71**, 48(1996)
25. O'regan, M. H., Phillis, J. W. and Walter, G. A. : The effect of the xanthine oxidase inhibitors, allopurinol and oxypurinol on the pattern of purine release from hypoxic rat cerebral cortex. *Neurochem. Int.*, **14**, 91(1989)
26. Kim, P., Yaksh, T. L., Burnett, P. C., Blum, M. R. and Sundt, T. M. : Cerebrospinal fluid levels of uric acid in dogs and the effect of allopurinol. *Brain Res.*, **402**, 87(1987)
27. Vatassery, G. T., Brin, M. F., Fahn, S., Kayden, H. J. and Traber, M. G. : Effects of high doses of dietary vitamin E on the concentration of vitamin E in several brain regions, plasma, liver and adipose tissue. *J. Neurochem.*, **51**, 621(1988)

(Received January 26, 1998)

The effect of chronic ethanol consumption on hepatic ultrastructure and function has been studied extensively by several investigators(11,12). Among various ultrastructural changes of hepatocytes induced by ethanol, megamitochondrial formation has attracted much attention because of its distinct morphologic changes. In addition, cup-shaped or extremely elongated mitochondria were frequently observed(13).

Therefore, this study was conducted to investigate the effect of dietary supplementation of vitamin A on the membrane property and ultrastructure of ethanol-administered rat livers.

MATERIALS AND METHODS

Animals and experimental diets

Male Sprague-Dawley rats were given pellet diet and tap water until they reached a weight of 130 to 150g. The animals were then divided into eight groups consisting of 10 rats, housed in individual stainless steel-bottomed cages. Then they were fed with experimental diets for 7 weeks. The diets contained different types of vitamin A which were β-carotene, retinyl acetate and retinoic acid(βE, RE, RAE group). Retinyl acetate(1.2mg/kg diet) was replaced by an equimolar concentration of β-carotene (7.2mg/kg diet) or retinoic acid(1.3mg/kg diet) except vitamin A-deficient group(FE group). The diet composition for experimental rats was used according to AIN-76 diet except for vitamin A level.

Feed was withdrawn overnight before receiving ethanol. A dose of 3.0g ethanol(30%, W/V)/kg B.W was injected to rats intraperitoneally. The rats of ethanol-free groups received 0.9% saline containing isocaloric sucrose instead of ethanol(FP, βP, RP, RAP group).

Plasma membrane preparation

Plasma membrane of rat livers was isolated by a modification of the method reported by Morré and Morré(14). Rat livers were homogenized using a polytron tissue homogenizer for 1 min in a medium containing 37mM tris malate buffer(pH 6.5) with 50mM sucrose, 1% dextran, 5mM $MgCl_2$, and 5mM mercaptoethanol. The homogenate was then centrifuged at 5000×g for 15 min to concentrate the plasma membrane. The supernatant and the top 1/3 of the pellet are removed for isolation of reference fraction. The middle one-third to one-half of the pellet was resuspended in 1mM $NaHCO_3$ and homogenized for 2 min at 600rpm using homogenizer. Another 5ml $NaHCO_3$ was added after the pellet was suspended. The mixture was centrifuged for 15 min at 5000×g. The supernatant was discarded and the light brown, top portion of the pellet was used for aqueous two-phase partition procedure. The final composition of the aqueous two-phase system was as follows. The concentrated membranes were mixed with 6.6%(W/W) dextran T-500(Pharmacia, Piscataway, NJ), 6.6%(W/W) polyethylene glycol 3350 (Fisher), 0.25M sucrose and 5mM potassium phosphate buffer, pH 7.2. The membrane fraction was resuspended in 1mM $NaHCO_3$.

The enrichment of plasma membranes was assessed by determining the activity of Na^+, K^+-ATPase as a marker enzyme. Ouabain-sensitive Na^+, K^+-ATPase activities were determined by a coupled kinetic assay as modified by Scharschmidt et al.(15).

Determination of the membrane fluidity

To determine the dynamic membrane response of the plasma membrane, electron spin resonance(ESR) spin labeling technique(16) was applied. 5-Doxylstearic acid (5-DSA, Aldrich chemicals) was used as a spin labeling agent. Sample of plasma membrane(4.2mg of protein/ml 1mM $NaHCO_3$) was added to the spin labeling agent that had initially been deposited on the side of the tube by evaporation from aqueous ethanol in a vacuum condition. ESR measurements were made using a Bruker×300-band spectrophotometer(Bruker×300, Germany). The spectra were recorded as the first derivative of the absorption curves, with field modulation of 100 kHz. The microwave power was held constant at 5.0mw. In all measurements, scanning time was 4 min, and modulation amplitude was 1.040 G. The order parameter value(S) for 5-doxylsteraric acid in the plasma membranes was estimated from the distances between the outer($2T_{II}$) and inner($2T_{\perp}$) hyperfine spectral splittings. The S value was calculated according to the formula of Gaffney(17).

$$S=\frac{T_{II}-T_{\perp}-C}{T_{II}-2T_{\perp}+2C}\times 1.723$$

where, $C= 1.4 - 0.053(T_{II} - T_{\perp})$

Electron microscopic examinations

A part of liver tissue was cut into small pieces, and

fixed with a 2.5% glutaraldehyde solution. Aldehyde -fixed samples were post fixed with 1% osmium tetroxide, dehydrated and embedded in 'EM bed 812'. Thin sections were cut on a ultramicrotome to 60~70nm, and stained with uranyl acetate and lead citrate(18). These samples were examined by transmission electron microscope (Hitachi-7100, Japan)

Statistical analysis

The significance of the differences between experimental groups was performed using SAS package by Dunkan's multiple test(α=0.05).

RESULTS AND DISCUSSION

Marker enzyme activity

The purity of the plasma membrane in rat livers was evidenced by measuring the specific activity of Na^+, K^+-ATPase as a maker enzyme. As shown in Table 1, Na^+, K^+-ATPase activity exhibited three times higher in plasma membrane than in liver homogenate.

Membrane fluidity

The term 'membrane fludity' has been broadly applied by biochemists and biophysicists to describe a number of phenomena related to the molecular structure and dynamics of the major components of biologic membranes. Membrane molecular order, or fluidity, is also influenced by membrane components such as proteins, cholesterol, and different phospholipid polar headgroups. These can interact with both the hydrophobic and hydrophilic portions of membrane lipids(19). Magnetic resonance techniques, in particular ESR, have been extensively used to study membrane physical structure in model and biological membranes(19).

Fig. 1 is the representative ESR spectrum of liver plasma membrane(LPM) labeled with 5-doxylstearic acid. Membrane fluidity in the present experiment was represented by order parameter (S value) as shown in Table 2. S value assumes a value between 0 and 1 representing a free fluid or immobilized environment,

Table 1. Na^+, K^+-ATPase activity as a marker enzyme of plasma membranes in rat liver

	Na^+, K^+-ATPase (nmol/min/mg protein)
Homogenate	5.3±0.9
Plasma membrane	16.4±2.1

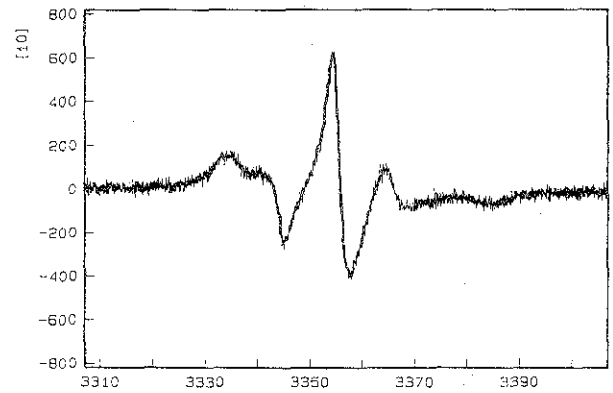

Fig. 1. ESR spectrum of liver plasma membranes labeled with 5-doxyl stearic acid.

respectively. LPM fluidity was decreased in rats fed vitamin A-deficient diet with ethanol as compared to control rats. The interesting thing is the alteration of membrane fluidity in response to a deficiency of vitamin A. Therefore, fluidity change of liver plasma membrane that ethanol had induced was influenced by dietary supplementation of vitamin A, but not influenced by the type of vitamin A. Although we did not analyze individual phospholipids fractions, deficiency of dietary vitamin A might exacerbate the change of membrane cholesterol/phospholipid ratio induced by ethanol administration. However, dietary supplementation of vitamin A seems to modulate this change to some extent.

Hamm et al.(20) found that microsomes from vitamin A-deficient animals exhibited a marked decrease in

Table 2. Effects of vitamin A and ethanol administration on membrane fluidity of liver plasma in rats

Group[1)]	S value
FE	$0.558\pm0.01^{ab2,3)}$
FP	0.530 ± 0.01^{c}
βE	0.560 ± 0.02^{ab}
βP	0.550 ± 0.02^{b}
RE	0.567 ± 0.01^{a}
RP	0.556 ± 0.02^{ab}
RAE	0.550 ± 0.02^{b}
RAP	0.548 ± 0.01^{b}

[1)]FE: Vitamin A-deficient group with ethanol
FP: Pair-fed vitamin A-deficient group without ethanol
βE: β-Carotene group with ethanol
βP: Pair-fed β-carotene group without ethanol
RE: Retinyl acetate group with ethanol
RP: Pair-fed retinyl acetate group without ethanol
RAE: Retinoic acid group with ethanol
RAP: Pair-fed retinoic acid group without ethanol
[2)]Means±S.D.
[3)]Values with the same superscript letter are not significantly different($p<0.05$)

membrane fluidity, by a 36% increase in the anisotropy parameter. The anisotropy parameter is directly related to membrane microviscosity and, hence, inversely related to the membrane fluidity. These authors also reported that no differences were observed in the cholesterol content, phospholipid content or cholesterol/phospholipid molar ratio of microsomal membranes. Therefore they have demonstrated that an altered membrane fluidity in liver microsomes from vitamin A-deficient animals was not related to the lipid composition *per se*.

The partition experiment results may be interpreted that β-carotene addition makes the membrane structure less compact and thus more spin probe molecules can enter directly the lipid phase. The membrane-fluidizing effect of β-carotene may be also important for its antioxidant activity(9,10). As reported by Cohn et al.(21), the efficiency of singlet oxygen quenching by β-carotene was inversely proportional to the solvent viscosity. Extrapolating these results to the membrane system one may conclude that the degree of fluidity should also affect the antioxidant efficiency of carotenoids as well as other membrane-localized antioxidants by modulating their diffusion rate within the membrane. The previous study in our laboratory observed that vitamin A supplementation, especially, retinyl acetate might partly reduce ethanol-induced lipid peroxidation and related damage(22).

Ethanol has been shown to enter the hydrophobic regions of the cell membrane causing an increase in membrane fluidity(23). The change in fluidity may result from a disturbance of the dynamic equilibrium of membrane phospholipids. However, the exact mechanism involved in the membrane-disordering effect of ethanol is unclear. According to Goldstein and Chin(24), the action of ethanol on biological membranes *in vivo* is of two types. In the acute type, the membranes become fluidized, whereas in the chronic type the membranes are rendered tolerant to the *in vitro* effect of ethanol.

Damage to plasma membranes is commonly associated with liver injury and vitamin A is well known to affect the stability of biological membranes(25). However, the

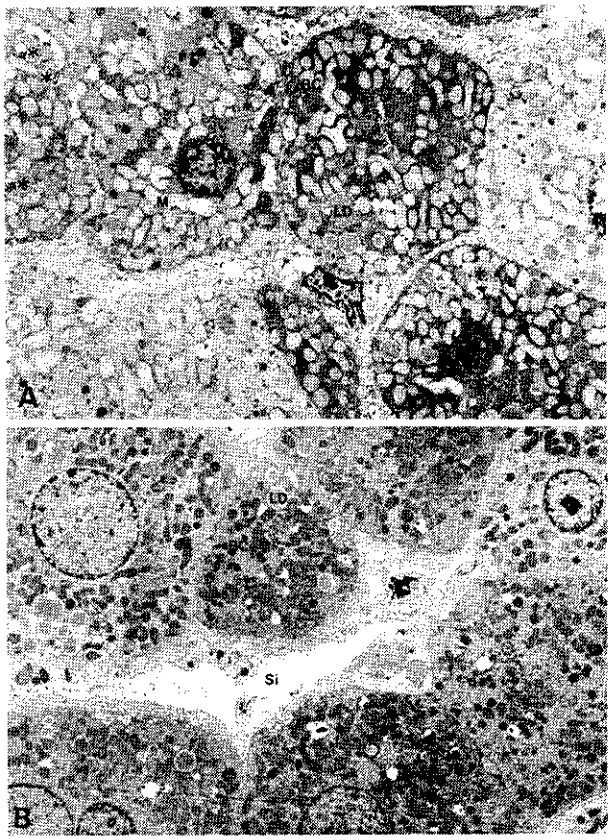

Fig. 2. Ultrastructural changes of hepatic tissue from rats fed with vitamin A deficient diet with ethanol(A) and without ethanol(B).
BC: bile canaliculus, LD: lipid droplet, M: mitochondria, Si: sinusoid (magnification×2,000)

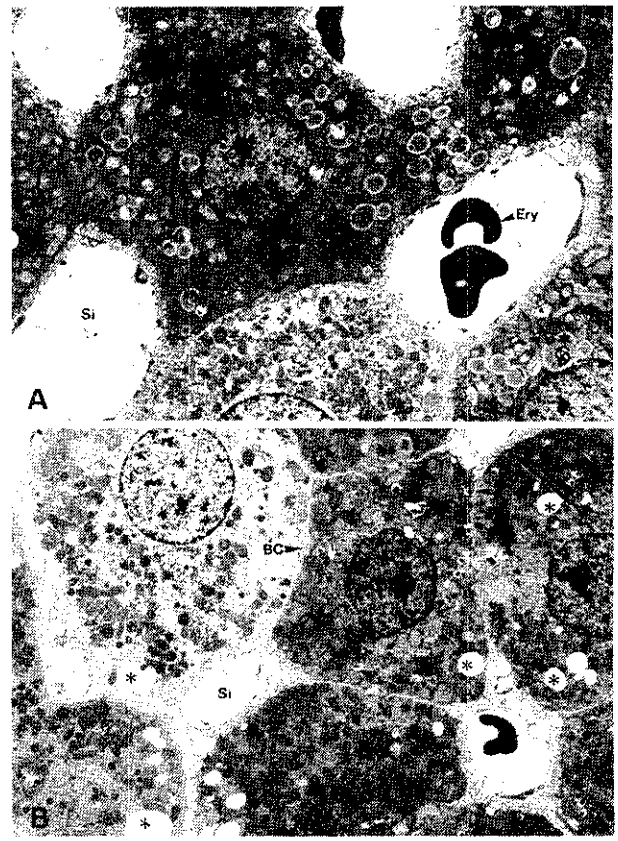

Fig. 3. Ultrastructural changes of hepatic tissue from rats fed with β-carotene supplemented diet with ethanol(A) and without ethanol(B).
BC: bile canaliculus, Ery: erythrocyte, LD: lipid droplet, Si: sinusoid (magnification×2,000)

interaction of ethanol with biological membranes is complex. It has not been proven that a single effect, such as an alteration in membrane fluidity is solely responsible for all functional alterations(19).

Morphological observations

The morphological lesions of rat livers were illustrated in Fig. 2~5. On the whole, the shapes of nucleus and rough-endoplasmic reticulum were preserved in every experimental rat. But the ultrastructural changes of hepatic mitochondria were distinct in some rats such as vitamin A-deficient rats with ethanol(Fig. 2). For example, hepatic mitochondria were mildly swelled in vitamin A-deficient rats with ethanol, because physiological balance of LPM might be changed. In rats fed with vitamin A-deficient or β-carotene diet with ethanol, lipid droplets were degenerated(Fig. 2, 3). Retinoic acid group with ethanol had electro-dense lipid droplets which appeared in the hepatocytes as well as in the Disse's space because of disruption of plasma membranes(Fig. 5).

Leo et al.(26) showed that in the baboon, the administration of ethanol together with β-carotene results in a more striking hepatic injury than with either compound alone. Also they observed the significant ultrastructural changes, including mitochondrial degeneration and markedly dilated membrane structures. In our study, β-carotene group with ethanol contained lipid droplets below 2μm, but much more droplets than retinyl acetate group with ethanol. The changes of bile canaliculi were not observed except retinoic acid group with ethanol.

Wakabayashi et al.(13) studied the effect of alkyl alcohols on the ultrastructure of mitochondria in the rat hepatocytes. They observed that the rats given ethanol for one month had cup-shaped or extremely elongated mitochondria.

In summary, acute ethanol administration induces the change of LPM fluidity in rats, especially in those fed a vitamin A-deficient diet. Although accurate mechanism involved in the plasma membrane-stabilizing effect of

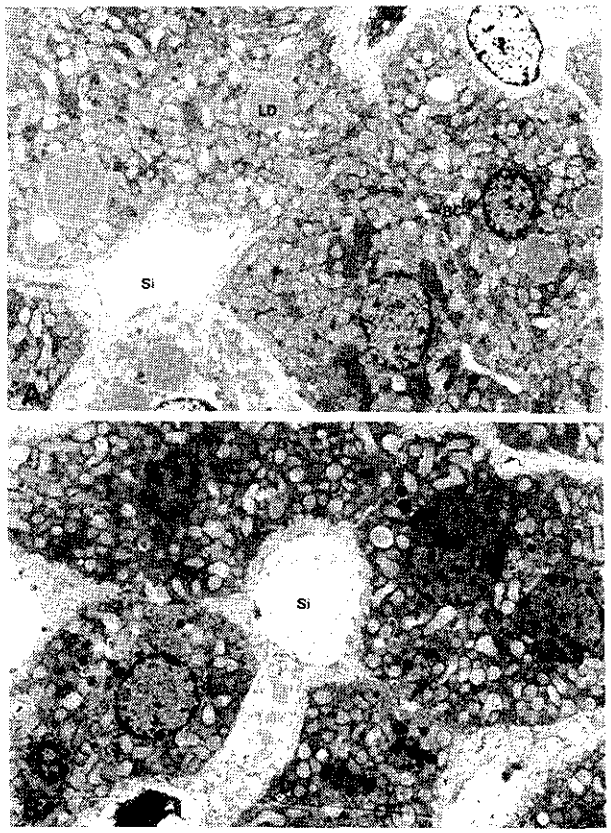

Fig. 4. Ultrastructural changes of hepatic tissue from rats fed with retyinyl acetate supplemented diet with ethanol(A) and without ethanol(B). BC: bile canaliculus, LD: lipid droplet, Si: sinusoid (magnification×2,000)

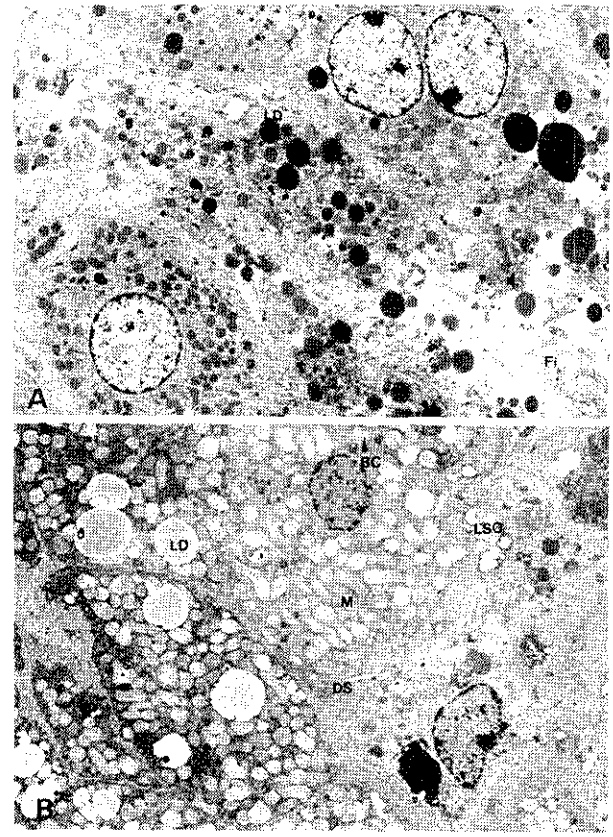

Fig. 5. Ultrastructural changes of hepatic tissue from rats fed with 13-cis-retinoic acid supplemented diet with ethanol(A) and without ethanol(B). BC: bile canaliculus, DS: disse's space, LD: lipid droplet, LSC: lipid storing cell, M: mitochondria (magnification×2,000)

vitamin A is still unclear, dietary supplementation of vitamin A such as retinyl acetate is needed to modulate this change. The direct involvement of LPM property on the cell damage caused by ethanol treatment remains to be established.

ACKNOWLEDGEMENTS

This work was supported by a grant No. KOSEF 941-0600-020-2 from the Korea Science and Engineering Foundation.

REFERENCES

1. Lieber, C. S. : Ethanol metabolism, cirrhosis and alcoholism. *Clin. Chim. Acta*, **257**, 59(1997)
2. Barry, R. E., Mcgivan, J. D. and Hayes, M. : Acetaldehyde binds to liver cell membranes without affecting membrane function. *Gut*, **25**, 412(1984)
3. Sivaprasadarao, A. and Findlay, J. B. C. : The interaction of retinol-binding protein with its plasma-membrane receptor. *Biochem. J.*, **255**, 561(1988)
4. Noy, N. : The ionization behavior of retinoic acid in lipid bilayers and in membranes. *Biochim. Biophys. Acta*, **1106**, 159(1992)
5. Wassall, S. R., Phelps, T. M., Albrecht, M. R., Langsford, C. A. and Stillwell, W. : Electron spin resonance study of the interaction of retinoids with a phospholipid model membrane. *Biochim. Biophys. Acta*, **939**, 393(1988)
6. Natsuki, R. : Effect of ethanol on phospholipid turnover and calcium mobilization in chick embryos. *Biochem. Pharmacol.*, **42**, 223(1991)
7. Kim, C. I., Leo, M. A., Lowe, N. and Lieber, C. S. : Differential effects of retinoids and chronic ethanol consumption on membrane in rats. *J. Nutr.*, **118**, 1097 (1988)
8. Jezowska, I. M., Wolak, A., Gruszecki, W. I. and Strzalka, K. : Effect of β-carotene on structural and dynamic properties of model phosphatidylcholine membranes. II. A ^{31}P-NMR and ^{13}C-NMR study. *Biochim. Biophys. Acta*, **1194**, 143 (1994)
9. Milon, A., Lazrak, T., Albrecht, A. M., Wolff, G., Weill, G., Ourisson, G. and Nakatani, Y. : Osmotic swelling of unilamellar vesicles by the stopped-flow light scattering method. Influence of vesicle size, solute, temperature, cholesterol and three α, ω-dihydroxycarotenoids. *Biochem. Biophys. Acta*, **859**, 1(1983)
10. Milon, A., Wolff, G., Ourisson, G. and Nakatani, Y. : Organization of carotenoids-phospholipid bilayer systems-Incorporation of zeaxanthin, astaxanthin, and their C-50 homologs into dimyristoylphosphatidylcholine vesicles. *Helv. Chim. Acta*, **69**, 12(1986)
11. Schilling, R. J. and Retz, R. C. : A mechanism for ethanol-induced damage to liver mitochondrial structure and function. *Biochim. Biophys. Acta*, **603**, 266(1980)
12. Taraschi, T. F. and Rubin, E. : Biology of disease. Effects of ethanol on the chemical and structural properties of biological membranes. *Lab. Invest.*, **52**, 120(1985)
13. Wakabayashi, T., Adachi, K. and Popinigis, J. : Effects of alkyl alchols and related chemicals on rat liver structure and function. 1. Introduction of two distinct types of megamitochondria. *Acta Pathol. Jpn.*, **41**, 405(1991)
14. Morré, D. J. and Morré, D. M. : Preparation of mammalian plasma membranes by aqueous two-phase partition. *Bio Techniques*, **7**, 946(1989)
15. Scharschmidt, B. F., Keeffe, E. B., Blankenship, N. M. and Ockner, R. K. : Validation of a recording spectrophotometric method for measurement of membrane-associated Mg^{-} and Na^{+}, K^{+}-ATPase activity. *J. Lab. Clin. Med.*, **93**, 790(1979)
16. Ogura, R., Sugiyama, M., Sakanashi, T. and Ninomiya, T. : ESR spin-labeling method of determining membrane fluidity in biological materials-Tissue culture cells, cardiac mitochondria, erythrocytes and epidermal cells-. *Kurume Med. J.*, **35**, 171(1988)
17. Gaffney, B. J. : Practical considerations for the calculation of order parameters for fatty acid or phospholipid spin labels in membranes. In "Spin labeling *theory and applications*" Vol. 1, Berliner, L. J.(ed)., Academic press, New York, p.567(1976)
18. Ernat, S. A. and Phalpott, C. W. : Preservation of Na^{+}-K^{+}-activated and mg^{++}-activated adenosine triphosphate activities of avian salt gland and teleost gill with formaldehyde as fixative. *J. Histochem. Cytochem.*, **18**, 251 (1970)
19. Taraschi, T. F. and Rubin, E. : Biology of disease. Effect of ethanol on the chemical and structural properties of biologic membranes. *Lab. Invest.*, **52**, 120(1985)
20. Hamm, M. W., Chan, V. and Wolf, G. G. : Liver microsomal membrane fluidity and lipid characteristics in vitamin A-deficient rats. *Biochem. J.*, **245**, 907(1987)
21. Cohn, P. F., Schalch, W. and Truscott, T. G. : The singlet oxygen and carotenoid interaction. *J. Photochem. Photobiol.*, **11**, 41(1991)
22. Yang, K. M. : Effects of dietary vitamin A on lipid perioxidation and membrane fluidity in ethanol-treated rat. Yeungnam University Ph. D. Thesis, p.52(1995)
23. Goldstein, D. B. : The effect of drugs on membrane fluidity. *Ann Rev. Pharmacol. Toxicol.*, **24**, 43(1984)
24. Goldstein, D. B. and Chin, J. H. : Interaction of ethanol with biological membranes. *Fed. Proc.*, **40**, 2073(1981)
25. Roles, O. A., Anderson, O. R., Lui, N. S. T., Shah, D. O. and Trout, M. E. : Vitamin A and membranes. *Am. J. Clin. Nutr.*, **22**, 1020(1969)
26. Leo, M. A., Kim, C. I., Lowe, N. and Lieber, C. S. : Interaction of ethanol with carotene : Delayed blood clearance and enhanced hepatotocixity. *Hepatology*, **15**, 883(1992)

(Received February 7, 1998)