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RESEARCH ARTICLE

Effect of polyunsaturated phosphatidyl-choline on lipid transport system in alcoholic liver injury

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Abstract

The aim of this study was to determine whether serum lipid composition and lipolytic activities in alcohol-induced liver dystrophy were modified by the co-administration of polyunsaturated phosphatidylcholine (PPC). Chronic alcohol intoxication was induced in rats by intragastric ethanol administration of 3.5 g/kg body weight per day over 56 days. Aqueous PPC suspension was given intragastrally in doses of 100 and 300 mg/kg body weight. Chronic alcohol intoxication led to the development of protein and lipid dystrophy of hepatocytes. PPC partially prevented alcoholic injury of the liver cells and had a normalizing effect on cholesterol esterification, lipolysis of lipoproteins and on the fatty acid composition of the main lipoprotein classes.

Introduction

Liver is the main organ in the body which determines the lipid transport system.[1] It is the site where the protein components essential for this system are synthesized (apoproteins, lecithin–cholesterol–acyltransferase (LCAT), HDL, VLDL, LDL receptors, chylomicron remnant receptor, circulating hepatic triglyceride lipase).[2] As the liver plays a central role in the metabolism of lipids, it is not surprising that liver diseases markedly affect plasma lipid and apolipoprotein levels.[3] Liver is the main organ of lipoprotein catabolism, oxidation and excretion of cholesterol.[1] Therefore, it is advisable to study the lipid transport system in alcoholic liver injury. At early stages alcoholic liver injury is manifested by fatty liver (steatosis) as a result of accumulation of lipids, mainly triglycerides, in hepatocytes. The lipid accumulation in liver is dependent upon a disproportion between triglyceride synthesis and secretion (VLDL). Fatty liver is associated with a deficiency in important nutritional factors: choline, essential fatty acids and polyunsaturated phospholipids.[4]

The main hepatoprotective properties of polyunsaturated phosphatidylcholine (PPC) are related to its membrane effects—stabilization of the membrane structures, normalization of the

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membrane-linked enzyme activities and regulation of the multiple metabolic and regenerative processes in the liver cells.[5] Studies of the effects of PPC on excretory/secretory hepatic functions in chronic alcohol intoxication resulting in protein and lipid dystrophy are of great theoretical and practical interest. An investigation of the serum components of the lipid transport system of hepatic origin seems therefore to be most appropriate.[6] The objectives of this study were to determine whether the lipid transport system, lipid composition and lipolytic activity in serum in alcohol induced liver lipid–protein dystrophy can be modified by the administration of PPC.

Materials and methods

Male white Wistar rats with an average body weight of 170 g were used. The animals were fed with balanced briquetted food and water *ad libitum*. Ethanol solution (25%) was given intragastrally in a dose of 3.5 g/kg body weight from 10–11 a.m. Aqueous PPC suspension was administered intragastrally in doses of 100 and 300 mg/kg body weight (from 3–4 p.m.) on the same day (PPC is a highly purified fraction of polyunsaturated phosphatidylcholine molecules from soy beans: 1-2-dilinoleoylphosphatidylcholine contribute approx. 50%[8]). Control animals received the same volume of water. The experiment lasted for 8 weeks. Animals were divided into four groups: 1, control animals; 2, animals receiving ethanol; 3, animals receiving ethanol+ PPC (100 mg/kg); 4, animals receiving ethanol+ PPC (300 mg/kg). One day after the last dose the animals were decapitated and hepatic tissue and blod serum were studied.

Thin slices of liver were fixed in neutral formalin and embedded in paraffin for staining with haematoxylin and eosin. Cryostate sections were prepared and stained with Sudan black B or Sudan III for the neutral lipid detection. The content of proteins (Lowry), triglycerides (by Fletcher using the standard Lachema kits) and cholesterol (Abel) were determined in liver homogenates. Lipid extraction was carried out according to Folch.[7]

The activity of the following lysosomal hepatic enzymes was assayed:

- acid lipase activity (3.1.1.3) was determined spectrophotometrically using beta-naphthylcaprilate (Sigma, USA) as substrate;[8]
- acid cholesterolesterase (1.1.1.13) was determined with 12.7 mkmol cholesterol-[1-^{14}C]-oleate (specific radioactivity 56 mCi/mkmol, Amersham, Great Britain) dispersed in lecithin–digitonin as substrate;[9,10]
- acid phospholipase A_1 (3.1.1.32) and phospholipase A_2 (3.1.1.4) activity were determined radiometrically with 1-acyl-2[1-^{14}C]-oleyl-glycero-3-sn-phosphorylcholine as substrate.[11,12]

The total cholesterol and of HDL, VLDL, LDL cholesterol (lipoprotein fraction were obtained by heparin precipitation in the presence of Mn^{2+}). The atherogenic index was calculated according to the formula:

$$\frac{\text{Total cholesterol} - \text{HDL cholesterol}}{\text{HDL cholesterol}}$$

The fatty acid spectrum of the lipoproteins was determined by gas chromatography of methyl esters. The fatty acids were methylated with 1.75 M sulphuric acid in absolute methanol for 2 hours at 75°C. The fatty acid methyl ester analysis was performed using model 3700 of Chromatograph (Moscow, Russia). The gas chromatograph was equipped with an FID-detector. Analytical conditions were: column, glass, 1.5 m × 3 mm; packing 10% DEGS on Inerton-Super 100/120 mesh. Temperatures were: column 197°C, injector 250°C, detector 250°C. Carrier gases were: He 30 ml/min, hydrogen 30 ml/min, air 300 ml/min. Quantitative assessment was done with a computing integrator. Fatty acid peaks were identified by comparing the retention time with authentic standards (US Biochemical Corporation, USA). The results were expressed as mean percentages of total fatty acid methyl esters.

In addition, we determined the total content of phospholipids and their separate classes differentiating them by two-dimensional thin-layer chromatography with phosphorous analysis using Vaskowsky reagents.[13,14] The lecithin–cholesterolacyltransferase activity (LCAT) (2.3.1.43) was determined by the modification of Dobiasova[15] using disks impregnated with [^{14}C]-4-cholesterol (Amersham, Great Britain). The fractional and molar activities of the enzyme were determined. The free cholesterol content was evaluated enzymatically (Boehringer Mannheim GmbH kits, Germany). To determine the post-heparin lipolytic activity (PHLA) in serum, heparin (350 U/kg body weight) was given i.p. 15

Table 1. *Effects of polyunsaturated phosphatidylcholine on the hepatic cholesterol and triglycerides content and the lysosomal lipolytic enzymatic activity in chronic alcohol intoxication*

Index	Control (n= 10)	Ethanol (n= 10)	Ethanol+ PPC: PPC, 100 mg/kg (n= 10)	Ethanol+ PPC: PPC, 300 mg/kg (n= 10)
Cholesterol (mg/g)	2.16 ± 0.06	2.45 ± 0.08*	1.91 ± 0.05*†	1.86 ± 0.08*†
Triglycerides (mg/g)	10.6 ± 0.58	13.7 ± 0.38*	10.2 ± 0.6†	12.90 ± 0.91*
Protein (mg/g)	196 ± 7	181 ± 8	183 ± 7	180 ± 5
Acid lipase (mkmol/1 g·min)	0.88 ± 0.04	0.84 ± 0.03	0.88 ± 0.04	0.92 ± 0.03
Cholesterol esterase (nmol/1 g·min)	112 ± 6	113 ± 4	119 ± 9	107 ± 4
Phospholipase A_1 (mkmol/1 g·min)	3.20 ± 0.26	3.09 ± 0.10	3.38 ± 0.26	3.34 ± 0.24
Phospholipase A_2 (mkmol/1 g·min)	4.08 ± 0.41	5.56 ± 0.22*	4.08 ± 0.28†	4.60 ± 0.24†

The results are shown as means (± SEM). Statistically significant effects of PPC are represented by *$p<0.05$ (in comparison with control), †$p<0.05$ (in comparison with ethanol). PPC, polyunsaturated phosphatidylcholine.

minutes before decapitation. PHLA was determined radiometrically using an ultrasonic emulsion of glycerol-3-[1-^{14}C]-oleate (Amersham, Great Britain) and the postheparin hepatic triglyceridelipase activity (H-TGL) (3.1.1.32) was determined after lipoprotein lipase inhibition.[16]

The results are reported as mean± SEM. Statistical significance of differences between the experimental groups was assessed by Student's t-test. Significance was assumed at $p<0.05$.

Results

The data from the histological assessment showed lipid–protein dystrophy of liver after administration of ethanol for 56 days. This was confirmed by biochemical data demonstrating a significant elevation of liver triglycerides (by 29.2%) and cholesterol (by 13.4%) (Table 1) and by the histochemical data, indicating a considerable accumulation of neutral lipids in hepatocytes in the rats receiving ethanol. The majority of hepatocytes with abundant lipid inclusions were located in the perivenular and periportal zones of liver lobules and varied from 7% to 22% in different animals. The increase of hepatic triglyceride content is not so large as described by other authors, due to a lower dose of alcohol used.[17–19] Both doses of PPC (100 mg/kg and 300 mg/kg) prevented lipid–protein dystrophy of the liver.

At the same time, increased activity of phospholipase A_2 was revealed. The activity of the other hepatocyte lysosomal enzymes did not change and the protein content in hepatic tissue was at its lower normal limit. PPC decreased the cholesterol levels below the control values. PPC in a dose of 100 mg/kg body weight normalized the content of triglycerides and the activity of phospholipase A_2.

Chronic alcohol intoxication caused a decrease of total serum cholesterol due to a decrease of the VLDL cholesterol and triglycerides (Table 2). The proportion of sphingomyelin decreased and the content of palmitoleic acid increased in HDL composition. Chronic alcohol intoxication is usually accompanied by essential fatty acids deficiency and is characterized by decreased linoleic and linolenic acid levels and increases monoenoic fatty acids and their derivatives.[20] PPC in a dose of 100 mg/kg enhanced the hypocholesterolaemic effects of alcohol due to a decrease in LDL cholesterol with a non-significant increase in the triglyceride content (Table 2). PPC in a dose of 300 mg/kg normalized the total serum cholesterol due to an increase in the HDL and VLDL cholesterol, but with a decrease in the LDL cholesterol level. The redistribution of cholesterol among the main classes of lipoproteins led to a decrease of the atherogenic index. PPC in dose 300 mg/kg normalized the sphingomyelin fraction and palmitoleic acid in HDL.

The long-term use of ethanol decreased the content of essential fatty acids (linolenic and arachidonic acid) and increased the content of palmitoleic and eicosatrienoic acids in apo-B-containing lipoproteins. PPC normalized the spectrum of fatty acids in the apo-B-containing lipoproteins. A larger effect was obtained at the dose of 300 mg/kg PPC body weight.

Chronic alcohol intoxication of rats resulted in a decrease of the activity of LCAT and enzymes of

Table 2. *Effects of polyunsaturated phosphatidylcholine on the contents of lipids and on the composition of lipoproteins in blood in chronic alcohol intoxication*

Index	Control (n= 10)	Ethanol (n= 10)	Ethanol+ PPC	
			PPC, 100 mg/kg (n= 10)	PPC, 300 mg/kg (n= 10)
Total cholesterol (mmol/1)	1.65 ± 0.06	1.44 ± 0.05*	1.28 ± 0.05&	1.50 ± 0.05
Triglycerides (mmol/1)	0.84 ± 0.02	0.53 ± 0.01*	0.66 ± 0.05*†	0.70 ± 0.05*†
Atherogenic index	1.71 ± 0.13	2.15 ± 0.26	1.21 ± 0.10*†	0.95 ± 0.06*†
HDL composition:				
Cholesterol (mmol/1)	0.61 ± 0.04	0.51 ± 0.04	0.58 ± 0.02	0.77 ± 0.03*†
Phospholipids (mmol/1)	1.46 ± 0.21	1.58 ± 0.21	1.53 ± 0.18	1.65 ± 0.10
Lysophosphatidylcholine (%)	32.3 ± 1.6	32.8 ± 2.8	37.1 ± 3.9	31.9 ± 2.7
Sphingomyelin (%)	16.5 ± 1.6	12.1 ± 0.9*	11.6 ± 1.5*	15.5 ± 1.0†
Phosphatidylcholine (%)	34.1 ± 2.6	36.9 ± 4.1	36.8 ± 4.2	37.4 ± 1.6
Phosphatidylethanolamine (%)	8.8 ± 1.2	9.8 ± 2.3	6.3 ± 2.8	6.2 ± 1.8
Polyglycerophosphates (%)	8.3 ± 2.0	8.8 ± 2.1	8.3 ± 1.7	8.9 ± 1.9
HDL fatty acid (%)				
16 : 0	44.5 ± 7.1	30.2 ± 2.1	29.6 ± 2.6	34.0 ± 4.5
16 : 1	5.6 ± 0.9	8.2 ± 0.7*	6.2 ± 1.1	4.2 ± 0.6†
18 : 0	15.9 ± 1.5	19.5 ± 3.2	14.6 ± 1.1	15.0 ± 0.6
18 : 1	13.5 ± 0.6	15.4 ± 1.3	16.2 ± 1.6	14.6 ± 1.2
18 : 2	16.4 ± 1.1	12.8 ± 2.2	12.2 ± 1.2	15.5 ± 0.5
20 : 3	2.8 ± 0.6	3.6 ± 0.4	4.2 ± 0.6	3.6 ± 0.7
20 : 4	10.6 ± 1.9	10.3 ± 1.5	13.8 ± 2.0	15.7 ± 1.8
VLDL cholesterol mmol/1)	0.37 ± 0.01	0.24 ± 0.01*	0.30 ± 0.02*	0.32 ± 0.03†
LDL cholesterol (mmol/1)	0.67 ± 0.05	0.75 ± 0.05	0.46 ± 0.01*†	0.40 ± 0.03*†
VLDL+ LDL fatty acid (%)				
16 : 0	23.7 ± 1.3	26.7 ± 5.0	27.3 ± 5.1	20.0 ± 3.3
16 : 1	2.8 ± 0.3	5.2 ± 0.5*	3.5 ± 0.7	3.0 ± 0.4†
18 : 0	12.8 ± 1.0	13.3 ± 1.2	15.9 ± 3.1	15.5 ± 2.2
18 : 1	21.6 ± 1.3	18.9 ± 1.8	17.1 ± 2.7	16.1 ± 2.1
18 : 2	20.3 ± 2.3	13.9 ± 2.34*	14.9 ± 1.9*	18.1 ± 2.29†
20 : 3	2.1 ± 0.2	4.2 ± 0.7*	3.7 ± 0.6	2.5 ± 0.3†
20 : 4	16.8 ± 1.6	12.8 ± 1.2*	16.6 ± 3.1	25.1 ± 4.9*†

The results are shown as means (± SEM). Statistically significant effects of PPC are represented by *$p<0.05$ (in comparison with control), †$p<0.05$ (in comparison with ethanol). PPC, polyunsaturated phosphatidylcholine.

the lipolytic transformation of lipoproteins (Table 3). PPC normalized the activity of these enzymes in serum. Thus, PPC had a normalizing effect on the enzymatic systems of cholesterol esterification, the lipolytic transformation of lipoproteins and the composition of fatty acids of the main lipoprotein classes in chronic alcohol intoxication that led to the development of protein and lipid dystrophy of hepatocytes.

Discussion

Polyunsaturated phosphatidylcholine (PPC) attentuaes hepatic fibrosis induced by CCl_4 and human albumin in rats, and also accelerates the regression of pre-existing fibrosis.[21] These results are in keeping with previous findings, that PPC attenuates alcohol-induced fibrosis in the baboon.[22,23] Whereas septal fibrosis and transformation of their lipocytes into transitional cells developed in seven of the nine baboons fed the regular diet with ethanol, septal fibrosis did not develop in any animals fed PPC. They did not progress beyond the stage of perivenular fibrosis (sometimes associated with pericellular and perisinusoidal fibrosis) and had a significantly lesser activation of lipocytes to transitional cells.[23]

The early stages of alcohol liver injury are of special interest. The present study assessed liver in rats receiving (intragastrally) alcohol in dose 3.5 g/kg body weight for 56 days: the histological pattern of the liver; the activity of lysosomal enzymes and the liver lipids; the spectrum of serum lipoproteins and their chemical composition; and the enzymes of the esterification of cholesterol and lipolytic transformation of lipo-

Table 3. *Effects of polyunsaturated phosphatidylcholine (PPC) on esterification of the cholesterol and lipolytic activity in serum in chronic alcohol intoxication*

Index	Control (n= 10)	Ethanol (n= 10)	Ethanol+ PPC: PPC, 100 mg/kg (n= 10)	Ethanol+ PPC: PPC, 300 mg/kg (n= 10)
Lecithincholesterol-acyltransferase (%)	7.19 ± 0.65	4.21 ± 0.46*	6.82 ± 0.56†	7.26 ± 0.68†
Lecithincholesterol-acyltransferase (mkmol/1·h)	82.3 ± 6.7	50.8 ± 6.2*	74.2 ± 4.8†	78.3 ± 6.9†
Postheparin lipolytic activity (mkmol/1·min)	199 ± 18	131 ± 11*	172 ± 12†	178 ± 14†
Hepatic triglyceride-lipase (mkmol/1·min)	79.2 ± 6.7	39.8 ± 5.6*	58.8 ± 5.2†	74.7 ± 7.2†

The results are shown as means (± SEM). Statistically significant effects of PPC are represented by *$p<0.05$ (in comparison with control) and †$p<0.05$ (in comparison with ethanol).

proteins in blood serum. Our histological and histochemical studies showed the signs of lipid–protein dystrophy mainly in the perivenular and periportal zones of liver lobules. The following causes of such localization of alcohol liver injury seem to be possible.

The contribution of free radicals and products of lipid peroxidation in alcoholic liver injury has been discussed actively.[24] Hydroxyethyl free-radicals are generated as side-products of cytochrome P4502E1 (CYP2E1)-mediated oxidation of ethanol and these radicals might participate in the development of alcohol liver injury by triggering immunotoxic reactions.[25] Endotoxins and other bacterial toxins from the gut are the most powerful stimulants of the release of mediators such as TNFα, IL1, IL6 and other cytokines or reactive oxygen species by Kupffer cells and the monocyte system.[26] Ethanol-induced liver disease usually starts in cells surrounding the centrilobular vein. Long-term-alcohol exposure leads to an up to 10-fold introduction of CYP2E1 and to significantly reduced activity of the Se-dependent glutathione peroxidase in this region. Since CYP2E1 is suspected to initiate lipid peroxidation via reactive oxygen radicals, this suggests that the unfavourable CYP2E1/glutathione peroxidase ratio in the centrilobular region is involved in the initiation of ethanol-induced liver disease in this liver region.[27]

Hepatic glutathione depletion after chronic alcohol consumption was found both in experimental animals and in humans. The ultimate precursor of cysteine (one of the three amino acids of glutathione) is methionine. Methionine must first be activated by S-adenosylmethionine by an enzyme which is depressed in alcoholic liver disease. This block can be bypassed by S-adenosylmethionine administration, which restores hepatic S-adenosylmethionine levels. S-adenosylmethionine also contributes to the methylation of phosphatidylethanolamine to phosphatidylcholine. The methyl-transferase activity involved is reduced by alcohol consumption, but this can be corrected and the hepatic phosphatidylcholine levels restored by the administration of PPC.[24]

These studies show that PPC in doses of 100 mg/kg and 300 mg/kg attenuated the development of lipid–protein dystrophy of the liver in rats fed ethanol, normalized the activity of acid phospholipase A_2, LCAT, post-heparin lipolytic activity and hepatic triglyceride lipase. These indexes can be used for evaluation of the therapeutic effect of PPC.

In alcohol liver injury, PPC in doses of 100 mg/kg and 300 mg/kg decreased liver cholesterol content, LDL cholesterol and triglycerides in serum as well as the atherogenic index compared with the controls. These indexes can be used for evaluation of the adequacy of the PPC pharmacodynamics in alcohol liver injury.

Thus, PPC appear to exert some relatively non-specific beneficial effects (possibly through membrane stabilization) that might also affect alcohol-induced liver injury.[5,21–24] However, the inhibition of alcohol-induced lipid–protein dystrophy demonstrated in the present study may also reflect a more specific action: PPC normalized the abnormal values of circulating LCAT and lipolytic enzymes.

References

1. Lopuchin YuM, Archakov AI, Vladimirov YuA, Kogan EM. Cholesterinosis. Moscow: Medicine, 1983.

2. Breslow Jan L. Genetic basis of lipoprotein disorders. Heart failure 1989;5:108–12.
3. Seidel D. Lipoproteins in liver diseases. J Clin Chem Clin Biochem 1987;115:321–31.
4. Dianzani MU. Reactions of the liver to injury. Fatty liver. In: Farber E, Fisher MM, editors. Toxic injury of the liver. Part A. New York and Basel: Marcel Dekker, 1979:281–329.
5. Gundermann K-J. The "essential" phospholipids as a membrane therapeutic. Szczecin: Jota, 1993.
6. Taskinen MR, Nikkila EA, Valimaki M, *et al.* Alcohol-induced changes in serum lipoproteins and their metabolism. Am Heart J 1987;113:458–64.
7. Folch J, Lees M. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–503.
8. Barrett AS. Lysosomal enzymes. Assay methods. In: Dingle JT, editor. Lysosomes: a laboratory handbook. Amsterdam: North-Holland Publications Company, 1972:110–25.
9. Pittman RC, Khoo SC, Steinberg D. Cholesterol esterase in rat adipose tissue and its activation by cyclic adenosine 3′,5′-monophosphate-dependent protein kinase. J Biol Chem 1975;250:4505–11.
10. Severson DL, Fletcher T. Characterization of cholesterol ester hydrolase activities in rabbit and guinea pig aortas. Atherosclerosis 1978;31:21–32.
11. Robertson AF, Lands WEM. Positional specificities in phospholipid hydrolysis. Biochemistry (Wash) 1962;1:804–10.
12. Stoffel W, Trabert V. Studies on the occurrence and properties of lysosomal phospholipase A_1 and A_2 and the degradation of phosphatidic acid in rat liver lysosomes. Hoppe-Seyler's Zeitschr Physiol Chem 1969;350:836–44.
13. Kates M. Techniques in lipidology. Isolation, analysis and identification of lipids. New York: American Elsevier Publications Company Incorporation, 1972.
14. Vaskowsky VE, Kastersky EJ, Vasenlin JM. A universal reagent for phospholipids analysis. J Chromatog 1975;114:129–41.
15. Dobiasova M. Lecithin: cholesterol acyltransferase and regulation of endogenous cholesterol transport. Adv Lipid Res 1983;20:107–94.
16. Baginsky ML, Brown WN. A new method for the measurement of lipoprotein lipase in postheparin plasma using sodium dodecyl sulfate for the inactivation of hepatic triglyceridelipase. J Lipid Res 1979;20:548–56.
17. Buko VU. Molecular mechanisms of hepatoprotective action of prostaglandins in alcoholic liver injury. Zeitschr Gastroenterol 1991;29:49–53.
18. Goheen S, Rearson E, Larkin E, Rao G. The prevention of alcoholic fatty liver using dietary supplements: dihydroxyacetone, pyruvate and riboflavin compared to arachidonic acid in pair-fed rats. Lipids 1981;16:43–51.
19. Segarnick D, Cordasco D, Agura V *et al.* Gamma-linolenic acid inhibits the development of the ethanol-induced fatty liver. Prostaglandins Leucot Med 1985;17:277–82.
20. Holman R. Control of polyunsaturated fatty acids in tissue lipids. Am J Clin Nutr 1986;5:183–211.
21. Xiaoli Ma, Jingbo Zhao, Lieber Ch.S. Polyenylphosphatidylcholine attenuates non-alcoholic hepatic fibrosis and accelerates its regression. J Hepatol 1996;24:604–13.
22. Lieber CS, Robins SJ, Li J *et al.* Phosphatidyl choline protects against fibrosis and cirrhosis in the baboon. Gastroenterology 1994;106:152–9.
23. Lieber CS, De Carli LM, Mak KM, Kim CJ, Leo MA. Attenuations of alcohol-induced hepatic fibrosis by polyunsaturated lecithin. Hepatology 1990;12:1390–8.
24. Lieber CS. Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. Adv Pharmacol 1997;38:601–28.
25. Albano E, Clot O, Bellomo G *et al.* Ethanol-derived free radicals and alcohol toxicity. Alcohol Alcohol 1997;32:SY58,323.
26. Bode JC. Endotoxin/cytokine interactions in alcoholic liver disease (ALD). Alcohol Alcohol 1997; 32:SY59,323.
27. Lindros KO. Alcohol metabolism in liver: zonation and pathology. Alcohol Alcohol 1997;32: SY106,335.