CLINICAL STUDY

Plasma lipid parameters in patients with alcoholic fatty liver after treatment with essential phospholipids

Turecky L, Kupcova V, Szantova M, Uhlikova E

Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava. **laco.t@seznam.cz**

Abstract

Background: **Fatty liver is the earliest and most common response to alcohol. The accumulation of lipid particles in hepatocytes alters the ultrastructure of cellular membranes.**
The purpose of our study was to investigate the effect of the administration of essential phospholipids on plasma lipid parameters in patients with alcoholic fatty liver.
Methods: **Our open clinical trial was performed in patients suffering from alcoholic fatty liver. The investigated group consisted of 29 patients. Two capsules of Essentiale forte were administered 3 times daily for 3 months. Individual biochemical parameters were examined each month. Values of total cholesterol, HDL- and LDL-cholesterol, triacylglycerols, apoprotein A and B were determined.**
Results: **The therapy with essential phospholipids had positive effects on the parameters of hepatocyte integrity. The levels of total cholesterol, triacylglycerols and apoprotein B were significantly higher in patients with fatty liver than in the controls. The concentration of HDL-cholesterol was also higher before the therapy than in the control group. There was no difference in levels of apoprotein A and LDL-cholesterol between the patients and the controls. There was no significant therapeutic effect on plasma lipid parameters in the group of patients with fatty liver.**
Conclusions: **The effects of treatment of alcoholic fatty liver with essential phospholipids were studied. The therapy had positive effects on the parameters of hepatocyte integrity. There was no significant therapeutic effect of the therapy on plasma lipid parameters.** ***(Tab. 2, Fig. 1, Ref. 20.)***
Key words: lipoproteins, cholesterol, fatty liver, essential phospholipids, Essentiale.

Alcoholism is an important public health problem faced by our society. Europe is the continent with the highest alcohol consumption in the world. The hepatotoxicity of ethanol has also been demonstrated in humans. Individuals with morphologically normal liver developed fatty liver when given ethanol, either in addition to a normal diet, or as an isocaloric substitution for carbohydrates in a variety of non-deficient diets (Lieber et al, 1965). Three common pathologic changes resulting from alcohol abuse are fatty liver, hepatitis and cirrhosis. About 50 % of alcoholics have a fatty liver, 20–30 % acute or chronic hepatitis and 10–20 % liver cirrhosis (Gundermann et al, 1995).

Fatty liver is the earliest and most common response to alcohol. The accumulation of lipid particles in hepatocytes alters the ultrastructure of cellular membranes. One of the basic components of biological membranes are represented by phospholipids. Phospholipids and the steric configuration of fatty acids which are bound in their molecules are responsible for "fluidity" of biological membranes. The fluidity of membranes is of essential importance for their function. There are many reports about positive therapeutic effects of essential phospholipids in the treatment of liver diseases. However there are relatively few reports on the effect of the administration of essential phospholipids on plasma lipids and lipoproteins in patients with liver diseases.

The purpose of our study was to investigate the effect of the administration of essential phospholipids on plasma lipid parameters in patients with alcoholic fatty liver.

Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava, and 3rd Department of Medicine, Derer's Hospital, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: L. Turecky, MD, PhD, Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, LFUK, Sasinkova 4, SK-811 08 Bratislava 1, Slovakia.

This work was supported by VEGA grant 01/9243/02.
The authors wish express their appreciation to E. Hederova for her technical assistance.

Material and methods

The open clinical trial was performed in patients suffering from alcoholic fatty liver. The investigated group consisted of 29 patients (17 men + 12 women), mean age 48.9 years (range 31–65 years) with histologically verified liver steatosis. Two capsules of Essentiale forte were administered 3 times daily (3 times 600 mg of essential phospholipids) during 3 months. The control group consisted of 42 healthy persons (students and blood donors), 30 men and 12 women, mean age 38.8 years, who showed no abnormalities on the basis of ordinary physical and laboratory tests.

Individual biochemical parameters were examined each month. In addition to the routine parameters, there were determined serum levels of total cholesterol, HDL-cholesterol, triacylglycerols, apoprotein A and B were determined. Cholesterol and triacylglycerols were determined by enzymatic methods (Cholesterol DST-P and Triglyceridy DST-P, DOT diagnostics s.r.o., Praha, Czech republic). HDL-cholesterol was determined after the precipitation of LDL+VLDL by magnesium chloride/phosphotungstic acid (HDL-C, Boehringer Mannheim GmbH, Germany). LDL-cholesterol was calculated according to Friedewald et al (1972). The quantification of apoproteins was performed by electroimmunoassay with monospecific antibodies (anti-apoA-I, OUED 08/09 and anti-apoB, OSAN 08/09, Behringwerke AG, Marburg, Germany) according to the procedure described by Laurell (1966). When the data had displayed the Gaussian distribution, parametric tests were used (Student's t-test for differentiating the averages and Pearson's correlation coefficient). When data had not had the Gaussian distribution, non-parametric tests were used (Wilcoxon's test for comparing populations and Spearman's correlation coefficient).

Results

The therapy with essential phospholipids had a positive effect on subjective symptoms of patients with liver steatosis. The therapeutic effect of the substance on the patient's well-being and the clinical condition were assessed as being good in 23 patients (76 per cent). There was a significant improvement in parameters of hepatocyte integrity (activities of aminotransferases) (Tab. 1). The decrease in enzyme activities was visible after one month of therapy, but this change was not statistically significant. The activities of both aminotransferases (ALT and AST) were significantly decreased two and three months after the beginning of the therapy. The activity of ALT was decreased by about 25 % and AST by about 33 % during the therapy. The activity of gamma-glutamyltransferase (GMT) was also significantly decreased after the therapy. There was no statistically significant change in the plasma level of albumin during the therapy but the albumin concentration of patients with fatty liver before the therapy had not been different from the control group. The same results were found for plasma enzyme activities of cholinesterase.

The main plasma lipid parameters – total cholesterol and triacylglycerols – were significantly higher in patients with fatty liver in comparison to the control group (Fig. 1). There was a slight decrease in levels of both investigated parameters during the therapy with essential phospholipids, but this change was not statistically significant. The concentration of HDL-cholesterol of patients before the therapy was significantly higher than that of the control group. There was no statistically significant difference between the levels of HDL-cholesterol before and after the treatment with essential phospholipids. Levels of LDL-cholesterol in the group of patients with fatty liver were slightly higher than in the control group, but this difference was not significant. Similar results were found also for apoprotein A. Plasma levels of apoprotein B were significantly higher in patients with fatty liver than in the control group of healthy individuals (Tab. 2). There was no significant difference in apoprotein B concentration before and after the therapy. There were very good correlations between the levels of apoprotein A and HDL-cholesterol ($r=0.86$, $p<0.001$), and apoprotein B and total cholesterol ($r=0.58$, $p<0.001$).

Discussion

Fat accumulation in the liver is the earliest and the most common response to alcohol. Fatty liver can be induced by either acute or chronic administration of alcohol both in laboratory animals and man (Lieber, 1985). Interactions of ethanol with lipids are multiple and complex. Lipids accumulating in the liver may originate from (a) dietary lipids, (b) adipose tissue as free fatty acids, or (c) they may be synthesized in the liver itself. Alcohol could (a) increase peripheral fat mobilization, (b) enhance hepatic triacylglycerols synthesis, (c) decrease lipid oxidation in the liver and (d) decrease hepatic lipoprotein release. Depending on conditions, any of the three sources and various mechanisms

Tab. 1. Results of routine hepatological laboratory tests.

Parameter	ALT µkat/l	AST µkat/l	GMT µkat/l	Cholin-esterase µkat/l	Albumin g/l
before therapy	0.81±0.06	0.68±0.07	2.05±0.38	71.4±5.2	41.8±2.3
after therapy	0.58±0.04	0.46±0.03	0.83±0.09	68.1±5.1	40.2±3.5
statistical significance	$p<0.05$	$p<0.05$	$p<0.01$	NS	NS

Results are given as mean±SEM, ALT – alanine aminotransferase, AST – aspartate aminotransferase, GMT – gamma-glutamyltransferase, NS – not significant

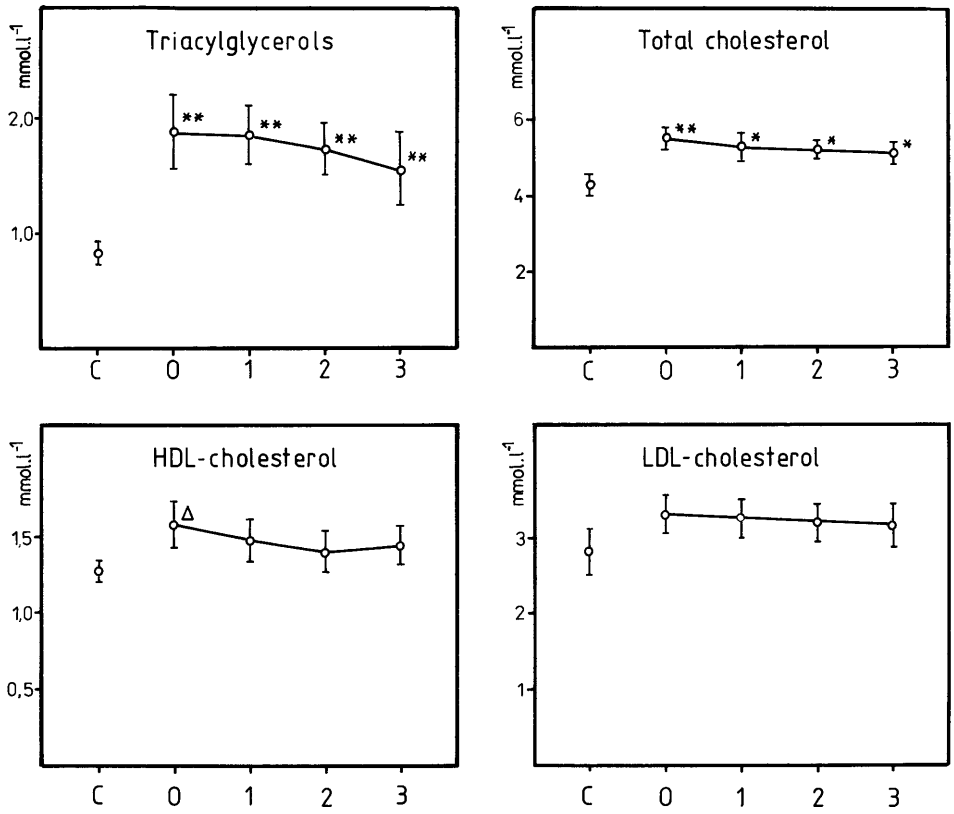

Fig. 1. Blood plasma lipid parameters in controls (C) and in group of patients during the therapy. 0 – before therapy, 1 – one month, 2 – two months, 3 – three months of therapy. Results are expressed as mean±SEM. Statistical significance of results in comparison to controls: ** p<0.001, * p<0.01, • p<0.05

can be implicated. Most of the patients with pure fatty liver are virtually asymptomatic. Typical abnormalities in laboratory tests are slightly or moderately elevated activities of gamma-glutamyltransferase (GMT) and aminotransferases (AST and ALT), as can also be seen in our group of patients with alcoholic fatty liver. Alcoholic steatosis is completely reversible in most instances (Lieber, 1996).

In primates, ethanol consumption also causes a decrease in liver concentration of phospholipids and phosphatidylcholine (PC). The phospholipid content of mitochondrial membranes is decreased with a significant reduction in the levels of PC (Arai et al, 1984), and associated with striking morphological changes. The alterations in the phospholipid composition of mitochondrial membranes appear to be responsible for the decrease in cytochrome oxidase activity and other biochemical alterations produced by long-term ethanol consumption. It was found out that the administration of phospholipid preparations rich in polyunsaturated PC or virtually pure polyunsaturated PC totally prevented alcohol-induced fibrosis and cirrhosis in primates (Lieber et al, 1994).

The main active constituent of essentiale is represented by "essential" phospholipids whose side chains carry predominantly highly unsaturated fatty acids. Their chemical structure is similar to that of endogenous phospholipids, to which they are superior in function because of their high content of essential fatty acids. The application of essential phospholipids with its high amount of polyunsaturated PC molecules, especially 1,2-dilinoleoyl-phospatidylcholine, has been widely accepted in the treatment of different liver diseases (Gundermann et al, 1995). The first double-blind study with alcoholic fatty liver dates as back as to 1979 (Knüchel, 1979).

The positive effect of essential phospholipids on patients with alcoholic fatty liver was found also in our study. Statistically significant improvements in ALT, AST and GMT values as well as subjective conditions were observed in our group of patients with alcoholic fatty liver after the treatment with essential phospholipids.

The consumption of alcohol has profound effects upon lipid and lipoprotein metabolisms not only in the liver but also in plasma. In experimental animal studies, the daily intake of ethanol caused a significant increase in serum cholesterol and triacylglycerols levels (Parkes et al, 1989). Increased levels of plasma triacylglycerols and total cholesterol in alcoholic patients with a non-cirrhotic liver injury were described by Duhamel et al (1984). Similar results – slight but statistically significant increase in plasmatic levels of triacylglycerols and total choles-

Tab. 2. Plasma apoproteins in patients with fatty liver before and during treatment with Essentiale forte.

Parameter	Controls	Before therapy	After		
			1 month	2 months	3 months
apoprotein A-I	1314±24	1316±72	1248±62	1230±53	1265±61
apoprotein B	902±40	1063±70*	1067±78*	1070±80*	1054±70*

Results are given as mean±SEM, * statistically significantly increased in comparison to controls ($p<0.05$)

terol were also found in our group of patients with alcoholic fatty liver. Another characteristic change typical for alcoholic fatty liver, namely the increase in plasma HDL-cholesterol, was found in our patients with fatty liver only before the beginning of treatment with essential phospholipids. Levels in HDL-cholesterol after the the beginning of therapy were not significantly different from controls. The decrease in HDL-cholesterol after the beginning of therapy could be the result of treatment with essential phospholipids, or the result of the cessation of alcohol consumption during the therapy. Gehrisch et al (1986) described the decrease in HDL-cholesterol in plasma of chronic alcoholics after 3 weeks of abstaining from ethanol.

In contrast to HDL-cholesterol, LDL-cholesterol stays unchanged during acute (Goldberg et al, 1984) or chronic ethanol administration (Crouse and Grundy, 1984). Also in our study, there was no significant change in the levels of LDL-cholesterol in patients with fatty liver in comparison to the control group. In spite of the unchanged level of LDL-cholesterol, the main cholesterol-containing lipoprotein in blood serum, the level of total cholesterol was increased in our group of patients. The increased concentration of total cholesterol could be explained by slightly increased levels of HDL-cholesterol and by increased levels of VLDL (significant increase in the triacylglycerols level). In their study, Duhamel et al (1984) showed that the content of cholesterol in VLDL of alcoholic patients with fatty liver was higher than in controls. The increased level of VLDL containing a higher amount of cholesterol in connection with the slight increase in HDL-cholesterol could be the cause of the significant increase in the concentration of total cholesterol in blood plasma of our patients with alcoholic fatty liver.

Many authors described that levels of apoprotein A-I and apoprotein A-II increase in alcoholic patients with liver steatosis (Mathurin et al, 1996; De Oliveira et al, 2000). In spite of the increased level of HDL-cholesterol in our patients before the beginning of therapy, the levels of apoprotein A-I were not significantly changed in any period of the study. This discrepancy could be explained by the fact, that the increase in HDL-cholesterol in our patients was relatively slight and that the alcohol consumption increases mainly the level of apoprotein A-II (Puchois et al, 1984).

The plasma level of apoprotein B was significantly increased in our group of patients with fatty liver in comparison to the controls. This increase in apoprotein B concentration could be explained by the increased level of VLDL in blood of these patients. It is known, that the consumption of alcohol increases the synthesis and secretion of VLDL (Duhamel et al, 1984; Hannuksela et al, 2002). The increased levels of VLDL containing apoprotein B as one of the major apoproteins in their molecule, could be responsible for the elevation of apoprotein B levels in plasma of our patients with alcoholic liver steatosis. This supposition is supported by the increased levels of plasma triacylglycerols, which are the main lipid component of VLDL.

There are controversial reports about hypolipidemic effects of essential phospholipids in literature (Bruneder and Klein, 1981; Klimov et al, 1995). The results of our study showed no hypolipidemic effect of essential phospholipids administration. None of the investigated lipid parameters were significantly decreased during the therapy. The differences between the levels of lipid parameters before and after the therapy with essential phospholipids were not significant. In view of these our results we cannot confirm any hypolipidemic effect of essential phospholipids in patients with alcoholic fatty liver.

References

Arai M, Gordon ER, Lieber CS. Decreased cytochrome oxidase activity in hepatic mitochondria after chronic ethanol consumption and the possible role of decreased cytochrome aa3 content and changes in phospholipids. Biochim Biophys Acta 1984; 797: 320—327.

Bruneder H, Klein HJ. Treatment of hyperlipoproteinaemia in diabetic patients. Dtsch Med Wchr 1981; 106: 1653—1656.

Crouse JR, Grundy SM. Effects of alcohol on plasma lipoproteins and cholesterol and triglyceride metabolism in man. J Lipid Res 1984; 25: 486—496.

De Oliveira E, Silva ER, Foster D, McGee Harper M, Seidman CE, Smith JD, Breslow JL, Brinton EA. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. Circulation 2000; 102: 2347—2352.

Duhamel G, Nalpas B, Goldstein S, Laplaud PM, Berthelot P, Chapman MJ. Plasma lipoprotein and apolipoprotein profile in alcoholic patients with and without liver disease: On the relative roles of alcohol and liver injury. Hepatology 1984; 4: 577—585.

Friedewald WT, Levy RJ, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499—509.

Gehrisch S, Richter U, Trubsbach A, Tappert C, Koritsch HD, Jaross W. Das verhalten von lipoproteinen und apolipoproteinen im blutserum bei chronischen alkoholikern nach alkoholentzug. Z Klin Med 1986; 41: 2133—2136.

Goldberg CS, Tall AR, Krumholz S. Acute inhibition of hepatic lipase and increase in plasma lipoproteins after alcohol intake. J Lipid Res 1984; 25: 714—720.

Gundermann KJ, Schneider E, Schumacher R, Janz B. The application of „essential" phospholipids (EPL) in alcoholic fibrogenesis — a key indication for future? Prog Hepato-Pharmacol 1995; 1: 180—190.

Hannuksela ML, Liisanantti MK, Savolainen MJ. Effect of alcohol on lipids and lipoproteins in relation to atherosclerosis. Crit Rev Clin Lab Sci 2002; 39: 225—283.

Klimov AN, Konstantinov VO, Lipovetsky BM, Kuznetsov AS, Lozovsky VT, Trufanov VF, Plavinsky SL, Gundermann KJ, Schumacher R. „Essential" phospholipids versus nicotinic acid in the treatment of patients with type IIb hyperlipoproteinemia and ischemic heart disease. Cardiovasc Drugs Ther 1995; 9: 779—784.

Knüchel F. Double-blind study in patients with alcoholic fatty liver. The influence of „essential" phospholipids on enzyme behaviour and lipid composition of the serum. Med Welt 1979; 30: 411—416.

Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Analyt Biochem 1966; 15: 45—52.

Lieber CS. Alcohol and the liver: metabolism of ethanol, metabolic effects and pathogenesis of injury. Acta Med Scand 1985; Suppl 703: 11—55.

Lieber CS. Pathogenesis and treatment of alcoholic liver disease. In: Lam SK, Paumgartner G, Wang B (Eds). Update on hepatobiliary diseases 1996. Dordrecht, Kluwer Academic Publishers, 1996, 69—84.

Lieber CS, Jones DP, DeCarli LM. Effects of prolonged ethanol intake: production of fatty liver despite adequate diets. J Clin Invest 1965; 44: 1009—1021.

Lieber CS, Robins S, Li J, DeCarli LM, Mak KM, Fasulo JM, Leo MA. Phospatidylcholine protects against fibrosis and cirrhosis in the baboon. Gastroenterology 1994; 106: 152—159.

Mathurin P, Vidaud D, Vidaud M, Bedossa P, Paradis V, Ratziu V, Chaput JC, Poynard T. Quantification of apolipoprotein A-I and B messenger RNA in heavy drinkers according to liver disease. Hepatology 1996; 23: 44—51.

Parkes JG, Hussain RA, Goldberg DM. Effect of alcohol on lipoprotein metabolism. I.High density lipoprotein binding. Clin Physiol Biochem 1989; 7: 269—277.

Puchois P, Fontan M, Gentilini JL, Gelez P, Fruchart JC. Serum apolipoprotein A-II, a biochemical indicator of alcohol abuse. Clin Chim Acta 1984; 185: 185—189.

Received June 25, 2003.
Accepted June 30, 2003.