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Antibodies Against Phospholipids and Oxidized LDL in Alcoholic Patients

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Summary

Antiphospholipid antibodies (APA) are a generic term describing antibodies that recognize various phospholipids. Hepatocyte damage is a cardinal event in the course of alcoholic liver injury and autoantibodies against phospholipids could play an important role in this process. APA in alcoholic patients seem to reflect membrane lesions, impairment of immunological reactivity, liver disease progression and they correlate significantly with disease severity. LDL oxidation is supposed to be one of the most important pathogenic mechanisms of atherosclerosis and antibodies against oxidized low-density lipoprotein (oxLDL) are some kind of an epiphenomenon of this process. The scope of our study was to determine some autoantibodies (IgG-oxLDL and antiphospholipid antibodies) and their possible changes in alcoholic patients. We studied IgG-oxLDL and four APA – anticardiolipin antibodies (ACA), antiphosphatidylserine antibodies (APSA) antiphosphatidylethanolamine antibodies (APE) and antiphosphatidylcholine antibodies (APCA) in 35 alcoholic patients with mildly affected liver function at the beginning of the abuse treatment. The control group consisted of 60 healthy blood donors. In the studied group, we obtained positive results concerning total ACA in 17.1 % of alcoholic patients (8.3 % in the control group), 11.4 % IgG-ACA (6.7 %), 8.6 % IgM-ACA (3.3 %), 14.3 % total APE (6.7 %), 14.3 % total APCA (8.3 %) and 20 % total APSA (8.3 % in the control group). The IgG-oxLDL (406.4±52.5 vs 499.9±52.5 mU/ml) was not affected in alcoholic patients. We conclude that the autoantibodies against oxLDL are present in sera of alcoholics and healthy blood donors. Based on our results which revealed a wide range of IgG-oxLDL titres in the healthy population, this parameter does not appear to be very promising for the evaluation of the risk of atherosclerosis. Alcoholics with only mild affection of liver functions did not exhibit a significantly higher prevalence of all studied antiphospholipid antibodies (ACA, APSA, APE, APCA) which could lead to membrane lesions in these patients.

Key words
Antiphospholipid antibodies – Autoantibodies – Oxidized LDL – Alcoholics

Introduction

Cell membranes consist of a phospholipid bilayer and proteins, the phospholipids can be modified by oxidative stress and free radicals. Antiphospholipid antibodies (APA) are a generic term describing antibodies that recognize various phospholipids. Cardiolipin is the most commonly tested mixed phospholipid. Other important phospholipids in biological membranes are phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. APA occur in patients with systemic lupus erythematodes, thrombosis, neoplastic disease, infections, advanced age and in women with repeated spontaneous abortions (Asherson 1992, Hörkkö *et al.* 1996).

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Hepatocyte damage is a cardinal event in the course of alcoholic liver injury and autoantibodies against native and modified phospholipids could play role in this process.

Alcohol abuse changes lipid metabolism with increased HDL-cholesterol, triglycerides and synthesis of free fatty acids in the plasma. The antiatherosclerotic effect of low ethanol intake is widely discussed. Chronic alcohol intake changes the elasticity of membranes, which also depends on their higher cholesterol content (Steinberg 1991).

There is considerable interest in the relation between oxidative processes and the development of atherosclerosis. Lipoprotein particles LDL and VLDL in the blood circulation as well as membrane phospholipids can be the site of radical reaction and lipid peroxidation. LDL oxidation is supposed to be one of the most important atherogenic mechanisms. Modified LDL, e.g. by oxidation (oxLDL), is capable of loading macrophages with cholesterol much more effectively than native LDL (Lin *et al.* 1995, Berliner and Heinecke 1996).

Oxidized LDL are probably also immunogenic. Thus, antibodies against oxLDL are an epiphenomenon of lipid peroxidation processes and they are detectable in man (Craig *et al.* 1994).

The aim of our study was to determine some autoantibodies (IgG-oxLDL and antiphospholipid antibodies) and to reveal their possible changes in alcoholic patients.

Material and Methods

We studied IgG-oxLDL and four APA – IgG and IgM anticardiolipin antibodies (ACA), antiphosphatidylserine (APSA), antiphosphatidylethanolamine antibodies (APE), total antiphosphatidylcholine antibodies (APCA), in alcoholic patients (heavy drinkers) with slightly affected liver function at the beginning of their abuse treatment (N=35, mean age 42±9 years) in our psychiatric therapeutic unit. Alcoholics had no signs of autoimmune symptoms or any other diseases. The control group consisted of 60 healthy blood donors (mean age 40±8 years). All subjects gave their informed consent before participating in this study. The serum was freshly frozen and all samples were measured together.

Biochemical parameters were measured by standard assays on a biochemical analyzer Cobas Mira Plus (Roche AG, Switzerland) and IgG-oxLDL was determined by commercial ELISA kit (Biomedica).

Antiphospholipid antibodies were identified using ELISA based on techniques described by Harris (1990) with some modifications. A solution of purified phospholipids (Sigma Chemicals, St. Louis, MO) was placed in each well of the plate, allowed to dry and was blocked by 10 % adult bovine serum in phosphate buffered saline (ABS in PBS). The serum samples were placed in duplicate wells at 1:50 dilution in 10 % ABS in PBS. After incubation, the wells were washed and horse radish peroxidase-conjugated goat antihuman total Ig or IgM or IgG (SEVAC, CR) diluted 1:5000 in 10 % PBS was added. After washing, colour was developed by adding ortho-phenylenediamine with H_2O_2.

Antiphospholipid assays other than anticardiolipin are not standardized and there are no commercial standard positive sera available for calibration. In our assay each plate was run with a positive and a negative control as the standard. We determined the cut-off values using sera from 30 blood donors. An absorbance greater than or equal to 3 S.D. above the mean for that phospholipid and antibody isotype was considered positive.

The statistical significance was evaluated using the ANOVA and test of alternative distribution.

Table 1. Biochemical parameters of alcoholics

Parameter	Alcoholics N = 35	Controls N = 60
ALT (μkat/l)	0.61±0.40	0.52±0.32
AST (μkat/l)	0.52±0.20	0.43±0.26
ALP (μkat/l)	1.29±0.41	1.34±0.52
Amylase (μkat/l)	1.41±0.45	1.26±0.53
Bilirubin (μmol/l)	7.70±4.00	8.10±3.10
Albumin (g/l)	46.70±2.70*	48.30±3.10
Cholesterol (mmol/l)	5.63±1.23*	5.00±1.09
HDL cholesterol (mmol/l)	1.16±0.27*	1.30±0.31
LDL cholesterol (mmol/l)	3.50±1.15**	2.90±0.95
Triglycerides (mmol/l)	1.99±1.04**	1.47±0.84

*Data are expressed as means ± S.D., * $p<0.05$, ** $p<0.01$, compared to the controls (Student's unpaired two-tailed t-test).*

Results

There were no significant differences between the alcoholic and control group concerning alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin and amylase. The alcoholics had significantly higher values of gamma-glutamyl transferase (GMT) (0.74±0.95 μkat/l vs controls 0.35±0.30 μkat/l, $p<0.01$).

The following lipid parameters were altered in the alcoholics: increased total cholesterol (5.63±1.23 mmol/l vs controls 5.00±1.09 mmol/l, $p<0.05$),

triglycerides (1.99±1.04 mmol/l vs 1.47±0.84 mmol/l, $p<0.01$) and LDL-cholesterol (3.50±1.15 mmol/l vs 2.90±0.95 mmol/l, $p<0.01$). On the other hand, HDL-cholesterol (1.16±0.27 mmol/l vs controls 1.30±0.31 mmol/l, $p<0.05$) was diminished. The changes of LDL-cholesterol and HDL-cholesterol in alcoholic patients were at the borderline of the normal range (Table 1).

Liver function was affected only slightly (increased GMT) and lipid parameters were mildly elevated. The IgG-oxLDL (406.4±52.5 vs. 499.9±52.5 mU/ml) was not changed in alcoholic patients. The 95 % confidential interval of our control group was from 395 to 605 mU/ml.

Table 2 shows the prevalence of antiphospholipid antibodies in alcoholic patients. All studied antiphospholipid antibodies had a higher prevalence in alcoholic patients than in the control group, but the differences were not significant. One patient was positive in all types of APA and two patients were positive in 3 APA types.

Table 2. Prevalence (%) of antiphospholipid antibodies in alcoholics

APA	Alcoholics (N=35)	Controls (N=60)
Total ACA	17.1	8.3
IgG-ACA	11.4	6.7
IgM-ACA	8.6	3.3
Total APE	14.3	6.7
Total APCA	14.3	8.3
Total APSA	20.0	8.3

No significant differences (test of alternative distribution).

Discussion

LDL oxidation is a crucial step in the development and progression of atherosclerotic lesions. Autoantibodies against oxidized LDL were present in the plasma of most of patients with coronary atherosclerosis and they can also be detected before the onset of clinically relevant signs of the atherosclerotic disease in patients considered at risk (Maggi *et al.* 1993). The high titre of IgG-oxLDL autoantibodies was described in systemic lupus erythematodes, severe atherosclerosis and in preeclampsia.

Our 95 % confidential interval for IgG-oxLDL is similar to the Viennese study describing the titre of IgG-oxLDL in 12 000 healthy working subjects. The peak distribution was at 300 mU/mL, and two thirds of all samples were found within the range of 150–800 mU/ml. Young people have higher titres than elderly ones, so that a protective function of IgG-oxLDL should be considered (Lapin *et al.* 1996).

LDL particles from alcoholic patients without serious liver disease indicate the presence of oxidatively modified epitopes and acetaldehyde adducts. LDL of alcoholic patients has a lower vitamin E content, is chemically modified *in vivo*, and exhibits altered biological function. These changes in heavy alcoholics may render LDL more atherogenic and thereby may counter the antiatherosclerotic effects of moderate alcohol consumption (Lin *et al.* 1995).

Wehr *et al.* (1997) described higher levels of IgG reactivity against both native and ethylated LDL in individuals with alcoholic liver disease than in alcoholics without liver injury. The high level of IgG reactivity in alcoholics with liver diseases were observed against malondialdehyde-modified, methylated, acetylated and carbamylated LDL. A selective high antiethylated LDL IgG reactivity was observed in 11 % of control subjects.

Vaarala *et al.* (1993) reported cross-reactivity between antiphospholipid antibodies and antibodies to oxidized LDL. It is possible that there is an immunological link between membrane lesions and atherosclerotic processes.

APA has emerged as the object of intense clinical and scientific interest in a wide spectrum of diseases over the last decade and deserves further investigations in connection with alcoholic intoxication (Schved *et al.* 1994).

Alcoholic liver injury has been reported to be directed preferentially against the proteins of cell membranes, sparing the phospholipids. However, antiphospholipid antibodies against certain cell membrane phospholipids are known to be associated with a variety of diseases (Chedid *et al.* 1994). The prevalence of ACA occurred in 57 % of patients with alcoholic liver cirrhosis (Gervais *et al.* 1996). However, APA levels were also high in patients with alcoholic hepatitis without cirrhosis (Bird *et al.* 1994).

Antibody prevalence was found in 15 % of alcoholic patients with normal liver function, 31 % of alcoholic patients with abnormal liver function, 81 % in patients with alcoholic hepatitis or cirrhosis and was absent in nonalcoholic controls (n = 8). Twenty out of 41 patients with alcoholic hepatitis or cirrhosis had antibodies against several cell membrane phospholipids (i.e., APE, APSA). Both IgA ($p<0.01$) and IgM ($p<0.008$) APE correlated significantly with disease severity. Chedid *et al.* (1994) did not find APCA, what is contrary to our positive findings.

The prevalence of autoantibodies in patients with liver disease is higher than in patients with systemic diseases including systemic lupus

erythematodes, where the prevalence of APA is 39 % (Alarcón-Segovia *et al.* 1989).

A high prevalence of other autoantibodies – antinuclear antibodies – ANA (22 %) and either anti ds-DNA (60 %) or anti ss-DNA (60 %) antibodies was described in alcoholics by Laskin *et al.* (1990). These results suggest that autoimmune mechanisms may play an important role in the pathogenesis of alcoholic liver diseases in at least some patients.

Antiphospholipid antibodies in alcoholic patients seem to reflect membrane lesions, impairment of immunological reactivity, liver disease progression and they correlate significantly with disease severity (Chedid *et al.* 1994, Hörkkö *et al.* 1996).

We thus conclude that the autoantibodies against oxLDL are present in the sera of alcoholics and also in healthy blood donors. On the basis of our results showing a wide range of the titres of IgG-oxLDL in healthy individuals, this parameter is not very promising for evaluating the risk of atherosclerosis. Alcoholics with only mild affection of liver function had a higher, but not significant, prevalence of all studied antiphospholipid antibodies (APSA, ACA, APE, APCA) which could lead to membrane lesions in these patients.

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