Sialic Acid Content of Low-Density Lipoprotein (LDL) is a Marker of LDL Oxidation

DARKO ČERNE[1] & JANA LUKAČ-BAJALO[2]

[1]Institute for Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Njegoševa 4, 1525 Ljubljana, Slovenia, darko.cerne@kclj.si
[2]Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia, jana.bajalo@ffa.uni-lj.si

Abstract: Oxidative stress constitutes a significant etiological factor for mercury intoxication, therefore, development of assays for monitoring oxidative stress of an organism is reasonable. Recently it was *in vitro* shown that a decrease in sialic acid (SA) content of low-density lipoprotein (LDL) is only a primary step in a chain of multiple modifications, which finally yields oxidized LDL (ox-LDL). To test the hypothesis that SA content of LDL and oxidation of LDL are interrelated also *in vivo*, we measured SA content of LDL and concentration of ox-LDL in plasma of 20 apparently healthy men. An average SA content of LDL was 34.7 ± 1.8 nmol per mg of LDL protein and concentration of ox-LDL 7.24 ± 1.01 % of total LDL. Linear regression analysis revealed that lower SA content of LDL was related to higher percentage of ox-LDL from total LDL ($r = -0.7085$, $p = 0.0008$). Our study is the first evidence that desialylation and oxidation of LDL occurs simultaneously also *in vivo*. These results suggest that SA content of LDL may serve as a sensitive marker of LDL oxidation possibly useful for monitoring oxidative stress of an organism in mercury polluted environmental areas.

Key words: mercury, oxidative stress, low-density lipoprotein, sialic acid

INTRODUCTION

Current evidence strongly suggests that oxidative stress constitutes a significant etiological factor for mercury intoxication[1,2], therefore, development of assays for monitoring oxidative stress of an organism is reasonable.

Oxidation of lipids and proteins substantially modifies the physical, chemical and immunological properties of low-density lipoprotein (LDL). Most recently, desialylation of LDL, e.g. loss of sialic acid (SA) residues on apo B and glycolipids, was shown *in vitro* to be an initial step in a chain of multiple modifications, which finally yields oxidized LDL (ox-LDL)[3,4]. Thus, the decreased SA content of LDL might be a marker of the extent of LDL oxidation indicating oxidative burden *in vivo*.

To test the hypothesis that SA content of LDL and oxidation of LDL are interrelated also *in vivo*, we measured SA content of LDL and concentration of ox-LDL in the plasma of 20 apparently healthy men. This may be of help when choosing SA content of LDL for monitoring oxidative stress of an organism in mercury polluted environmental areas.

RESULTS AND DISCUSSION

Apparently healthy men were prospectively and consecutively enrolled from healthy volunteers willing to participate in the study. To be included in the study, subjects had to have 1) absence of diseases indicating excessive oxidative stress, such as atherosclerosis, diabetes, chronic renal failure, ischaemia reperfusion injury, rheumatoid arthritis or certain nervous system diseases as verified by detailed history and clinical examination 2) systolic and diastolic blood pressure ≤140 and ≤90 mmHg 3) to be nonsmoker and 4) to have serum concentration of C-reactive protein ≤ 1,5 mg/L.

SA content of LDL was determined in isolated LDL fraction with ion-exchange chromatography as previously described[5]. Briefly, plasma containing 4.42 mM EDTA was used for LDL isolation. Butylated hydroxytoluene and Trolox (Hoffmann La Roche) were added at a final concentration of 20 μM of each immediately after centrifugation of blood cells. LDL was isolated by two-step density gradient ultracentrifugation at 100 000 rpm for two hours and with TLA 100.4 rotor in Optima™ TLX ultracentrifuge at 10 °C (Beckman). Lipoprotein(a) (Lp(a)) in the isolated LDL fraction was removed by immunoaffinity gel containing polyclonal anti-apo(a)-antibodies isolated from N Antiserum Lp(a) (Behring). Electrolytes in isolated LDL fraction free of Lp(a) were further removed using a gel filtration (Biogel P-6DG, Bio Rad). Desalted LDL fraction free of Lp(a) was concentrated three times by ultracentrifugation at 100 000 rpm for 75 minutes with rotor TLA 120.2 in Optima™ TLX ultracentrifuge (Beckman) at 10 °C. The protein concentration was determined by the method of Lowry *et al*. Desalted and concentrated LDL free of Lp(a) was diluted with 10 mM Tris pH 7.4 and sulphuric acid to a final concentration of proteins of 0.88 g/L in 0.02 M sulphuric acid. The hydrolyzing solution was incubated for one hour at 80 °C with permanent shaking and stored afterwards at –70 °C until further analysis. SA was determined in one batch at the end of the study with Aminex HPX-87

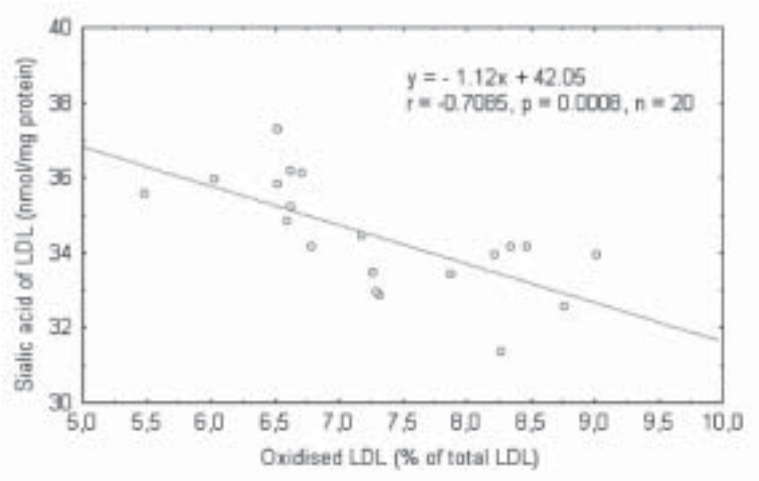

Figure 1. Correlation between the salic acid content of low-density lipoprotein (LDL) and the percentage of oxidized LDL in apparently healthy men.

cation-exchange resin column 300 x 7.8 mm (Bio Rad) and Micro-guard cation precolumn at 42 °C, a mobile phase of 0.003 M sulphuric acid at a flow-rate of 0.65 ml/min and UV detection at 206 nm. Oxidized LDL was determined in isolated LDL fraction, free of Lp(a), as previously described[6].

An average SA content of LDL was 34.7 ± 1.8 nmol per mg of LDL protein and concentration of ox-LDL 7.24 ± 1.01 % of total LDL. Linear regression analysis revealed that the lower SA content of LDL was related to higher percentage of ox-LDL from total LDL ($r = -0.7085$, $p = 0.0008$). Results obtained are graphically presented in Figure below.

A possible link between desialylation and oxidation of LDL was studied *in vitro* for the first time by Chappey *et al*. in 1998[3]. They found that LDL exposed to dialyzing buffer containing low EDTA concentration revealed to the partial oxidation, as assessed by the decrease in vitamin E and the increase in TBARS. At the same time SA content of LDL markedly decreased, which was concluded to be related to a subsequent alteration of lipoprotein integrity. Desialylation and oxidation of LDL was completely inhibited by concentration of EDTA in the dialyzing buffer above 1 mmol/L. At the same time Tertov *et al.*[4] reported a significant fall in the lipoprotein SA level after only one hour of incubation of native LDL with an autologous plasma-derived serum. While SA of LDL was continuously decreasing, LDL gradually revealed oxidative properties such as the increase of negative charge and susceptibility to copper-oxidation, the loss of alpha-tocopherol and the decrease of particle size. Thus, the desialylation of LDL was only the earliest event in a chain of multiple modifications, which finally yields highly ox-LDL.

CONCLUSIONS

We found a significant correlation between the decreased SA content of LDL and the increased percentage of ox-LDL from total LDL, which is in accordance with data previously published[3,4]. However, our result is also the first evidence that desialylation and oxidation of LDL occur simultaneously also *in vivo*. This result suggests that SA content of LDL may serve as a sensitive marker of LDL oxidation possibly useful for monitoring oxidative stress of an organism in mercury polluted environmental areas.

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