Phosphatidylcholine Hydroperoxide in HDL and nonHDL fraction

Aims

We tried to measure Phosphatidylcholine Hydroperoxide (PCOOH) in HDL and non-HDL fraction.

Methods

0.05 ml of 4 % sodium phosphotungustate and 0.05 ml of 0.5 M magnesium chloride (final concentration: sodium phosphotungustate 0.5 % magnesium chloride 0.05 M) are added to 0.5 ml serum with through vortex-mixing after addition of each reagent. The mixture was centrifuged at 3000 rpm for 15 min at 4°C after 20 min on ice. The supernatant was HDL and the precipitant was non-HDL. The precipitant was redissolved in 0.3 ml of distilled water. The total lipids were extracted by the Bligh and Dyer method for assay of lipid hydroperoxide.

CL-HPLC

HPLC column: SIL-NH2

Mobile phase: 2-propanol-methanol-water (135:45:20, v/v/v)

CL reagent: 10 mg/L Cytochrome *c* and 1.0 mg/L luminol in borate buffer (pH 10.0)

Standard: Photo-oxidized L-α-phosphatidylcholine, β-oleoyl-γ-palmitoyl（C18:1,[cis]-9/C16:0, SIGMA）

Detector: CLA-2100, CLA-FL2

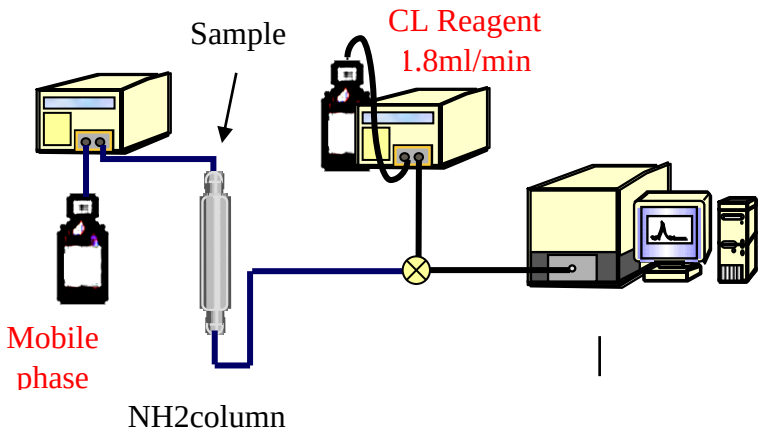

Fig. 1 CL-HPLC system

Result

PCOOH levels of serum, HDL and non-HDL fraction were measured for some samples (n= 72). Sum of PCOOH in HDL and non-HDL was 123±45% of serum. Therefor non-HDL PCOOH was calculated by the difference between serum- and HDL-PCOOH levels.

This data showed approximately 50% of serum phsophatidylcholine hydroperoxide was present in HDL fraction

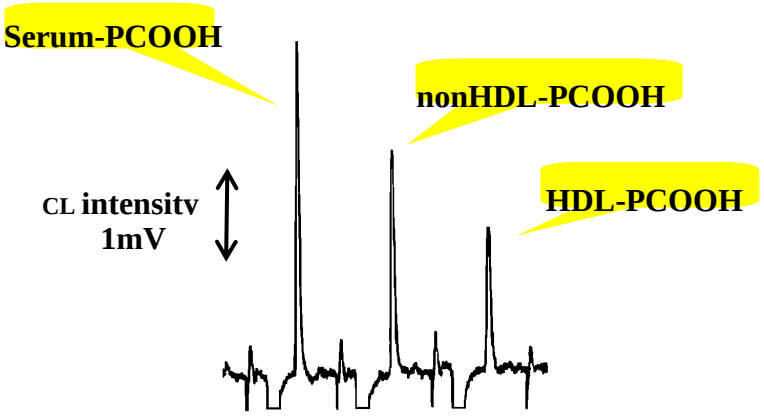

Fig. 2. CL-HPLC chromatogram of serum-PCOOH, nonHDL-PCOOH and HDL-PCOOH.

Reference

Nagashima, T., Oikawa, S. and Yamada, R. : Diabetes Research and Clinical Practice, in press