Application of vibrational spectroscopy in clinical research of human blood lipids and lipoproteins

Abstract

Metabolic syndrome is a clinical state, which characterizes simultaneous occurrence of abdominal obesity, increased blood pressure, disturbed carbohydrate and lipid metabolism with elevated concentration of low density lipioprotein (LDL) and reduced high density lipioprotein (HDL). This factors lead to atherosclerosis, what can be followed by myocardial infarction or ischemic stroke – the most common death cause according to the Eurostat data.

Standard diagnostics of dyslipidemia includes only basic lipidograms, within which total cholesterol, LDL "bad" cholesterol, HDL "good" cholesterol and triglycerides are determined. According to clinical trials, in case of dyslipidemia related with elevated TG level and cholesterol in the norm, these parameters do not give information about amount and modified structure of lipoproteins. Sometimes in patients, whose results are within normal limits, develops atherosclerotic changes, threatening their lives. Research show that this is result of presence of pathological subclasses of VLDL, LDL and HDL lipoprotein fractions.

Lipoproteins are made of triglycerides, cholesterol, phospholipids and proteins. The individual fractions differ from each other not only in the proportions of the listed components, but also in their function, in the size of the particles and their density. Disorders related with composition of lipoproteins, like TG or PL content can promote arising pathological subclasses of lipoproteins or their aggregation, what lead to arthrosclerosis. Its development can also be promoted by presence of oxidation-damaged lipoproteins.

In metabolic syndrome increased concentration of fatty acids is also observed, what promote creation of large VLDL and small, dense LDL and HDL. Therefore it is important to determine free fatty acids (FFA), not carried by lipoproteins, but by albumin, which is universal transport protein for hydrophobic molecules.

NMR spectroscopy enables determination of concentration of lipoprotein specific subclasses. However, access to this technique is still quite limited due to high price of instrumentation. Goal of this thesis was to evaluate possibility of application of vibrational spectroscopy, especially FT-IR, but also Raman, in detection of blood serum lipids and lipoproteins pathobiology. This tasks are significant from the viewpoint of both biomedical engineering and medical diagnostics.

To accomplish this goal, FTIR-ATR and Raman spectroscopy was applied to determine amount of TG carried by VLDL fraction (TG-VLDL) directly in blood serum, without necessity of time-consuming fraction isolation with ultracentrifugation. Blood samples were collected from 31 patients and VLDL fraction was isolated form sera with ultracentrifugation. In isolated samples of VLDL fraction TG-VLDL was determined with a reference, colorimetric method. FTIR-ATR and Raman spectra of blond serum were recorded to create calibration models with application chemometric method of regression PLS. Finally, calibration models were successfully validated with the reference method creating possibility of fast, reagent-free determination of VLDL-TG directly in blood serum.

Another step was evaluation of PL content in VLDL fraction and its impact on VLDL aggregation susceptibility. In VLDL samples isolated from sera of 22 patients with ultracentrifugation method PL content was determined with a reference colorimetric method. PL content was determined with FTIR-ATR spectroscopy as well. VLDL aggregation was induced by vortexing. Progress of aggregation was evaluated spectrophotometrically as increase of extinction at 680 nm. Spectra of VLDL spectra were recorded after aggregation too. It was shown, that higher PL content promote higher aggregation susceptibility. After

aggregation changes of apolipoprotein (lipoprotein structural proteins) secondary structures content were observed.

FTIR-ATR spectroscopy was applied to examine oxidative changes in plasma lipids in a animal model (sheep polish merino). Oxidative changes were simulated by giving Animals in to hemodialysis, leading to immune system activation and reactive oxygen species secretion by neutrophils during unspecific reaction. During lasting of hemodialysis blood samples were collected and plasma was isolated. Change of oxidatively damaged lipid metabolites was determinate with a reference method. Total lipid fraction was extracted from plasma to record FTIR-ATR spectra and determine spectral parameters related with oxidation. Research shown accordance between reference and FTIR-ATR methods and usefulness of vibrational spectroscopy in monitoring of oxidatively damaged lipids amount.

Impact of FA binding on albumin secondary structure was examined. Single particle of albumin, an universal transport protein, can bind up to 9 molecules of FA. Usually a molar ratio of FA to albumin fits in the range from 0.2 to 2.0 mol/mol. However, after meal, physical effort or during some metabolic diseases (like diabetes) this ratio can be higher. Change of amount of α-helices during increasing FA to albumin molar ratio was studied in the range from 0 to 20 mol/mol. Results were compared with crystallographic data available in the Protein Data Bank, which consists information about protein structures, including α-helices content, but only for pure protein or protein with fully saturated binding sites. Data shown rapidly increasing content of α-helices with ratio increasing from 0 to 2.0 mol/mol and relative structural stability from 2.0 to 9.0 mol/mol. This findings correspond with results obtained by molecular mechanics simulations. This study shown usefulness of FTIR-ATR spectroscopy in examination of FA amount bounded to albumin, nerveless research should be continued with blood serum or lipoprotein-deficient serum – a side product of ultracentrifugation.

Summing up, conducted study proved usefulness of vibrational spectroscopy in blood lipids and lipoproteins research. It can be successfully applied in evaluation of oxidative stress impact on lipids peroxidation caused by hemodialysis, TG-VLDL determination directly in blood serum without necessity of ultracentrifugation, PL content in VLDL and assessment of structural changes of proteins (VLDL apolipoproteins and albumin). It was proved, that higher PL content in VLDL fraction elevate its aggregation susceptibility.

In contrast to NMR, vibrational spectroscopy is widely available due to instrumentation lower cost. It does not need expensive reagents nor special measurement conditions, allowing rapid spectra recording of small volume samples. These advantages make vibrational spectroscopy convenient for clinical applications.