Identification of phospholipid-Apo B adducts in atherosclerosis by a mass spectrometry approach

Berichterstattung

Projektinformationen

ATHERO_MASS

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Final Report Summary - ATHERO_MASS (Identification of phospholipid-Apo B adducts in atherosclerosis by a mass spectrometry approach)

According to the World Health Organisation (WHO), cardiovascular diseases such as coronary artery

disease and stroke are still the major causes of mortality in EU countries, and consequently are responsible for a major portion of total healthcare costs (hospitalisation, medication, human resources, and productivity losses). To improve this situation, it is necessary to identify patients at high risk of vascular incidents before any clinical symptoms develop, thus allowing them to receive preventative treatment. This is turn requires better understanding of the mechanisms of disease development, and improved diagnostic markers for early stages of disease.

Coronary artery disease and stroke result from an underlying process called atherosclerosis, or hardening of the arteries. This is an inflammatory condition exacerbated by high circulating levels of low density lipoprotein (LDL), often referred to as 'bad cholesterol'. In fact, it is now understood that to cause atherosclerosis, the LDL must be chemically modified in the circulation or the wall of the artery, and one of the ways in which this happens is by oxidation, caused by damaging oxygen-containing compounds. LDL particles have lipid components (consisting of many different types of fats) and a protein component (ApoB-100); both of these are susceptible to damage by oxidation, and it is thought that both types of damage could contribute to the development of atherosclerosis. Therefore, it follows that these changes to LDL could have potential as markers for early stages of cardiovascular disease. In particular, damage to the protein either directly or by reaction with oxidised lipid molecules has been suggested to relate to disease, but the structure of these protein-oxidised lipids adducts has not yet been characterised. The best technique for studying the structure of oxidative modifications to LDL is mass spectrometry, which can measure the mass (weight) of many of the different molecules in LDL and determine if they have been modified by oxidation or if protein-oxidised lipids adducts have been formed. The technique is complex to apply, especially with ApoB-100 as it is an unusually big protein, and a comprehensive study this type of LDL oxidation and its occurrence in atherosclerosis had not previously been carried out.

The aim of the ATHERO_MASS project was to develop mass spectrometry technology to study the molecular makeup of LDL and detect many different types of LDL oxidation, but especially the formation of LDL protein-oxidised lipids adducts. The methodology was tested using normal LDL from healthy volunteers that had been treated artificially with oxidants to generate many different oxidised molecules. Once the methodology was established, the aim was to apply it to characterise LDL from patients with cardiovascular disease and compare it with LDL from healthy age-matched volunteers, to determine whether any of the components were different and had potential as diagnostic markers for disease. This latter part of the project was carried out in collabouration with Prof David Webb at the University of Edinburgh, United Kingdom. The first six months of the project was carried out at the University of Strathclyde, in Glasgow, United Kingdom, and the remainder at Aston University, Birmingham, United Kingdom.

During the project, advanced mass spectrometry techniques were developed to analyse the LDL particle. In the early stages of the project, methods to extract lipids from the LDL were tested and optimised, and the lipids present were identified by mass spectrometry. More than 350 different lipids were identified in LDL from healthy volunteers, some of which had not previously been reported in LDL. The methods developed were then applied to LDL from patients with chronic kidney disease, who have severe cardiovascular complications and incidence of atherosclerosis, and age-matched healthy people. Using chemometrics, a powerful statistical method, it was found that despite having similar general LDL lipid composition, there were significant differences in the levels of certain lipids in the disease and healthy

groups, specifically phosphocholines, triacylglycerols, sulfatides, plasmenyl-phosphatidylethanolamines and lipoaminos. In combination, these compounds were able to discriminate very well between disease and normal LDL, and hence are potential candidates to monitor disease at an early stage.

Using methods established during the project for preparing LDL protein and advanced mass spectrometry procedures, it was possible to 'map' a very large proportion of ApoB-100 (> 70 %). In view of the size of ApoB-100, this was an important achievement, which allowed the detection of several different types of oxidative damage to ApoB-100, including protein-oxidised lipid adducts. A new method for finding and characterising these oxidative modifications was developed, involving the use of a diagnostic fragment seen in the mass spectrum at m/z 184, which is characteristic for phospholipids, and this was used to detect the presence of oxidised phospholipid bound to ApoB-100 at a particular location (H2366) in LDL from both normal and disease groups. However, not all the disease samples had this modification, so further work is needed to investigate its relation to disease. In addition, development of the mass spectrometry methods allowed a wide range of oxidative and other modifications to ApoB-100 to be observed in clinical samples and to a lesser extent in controls. Some of these, such as changes in the sugar molecules attached to ApoB-100, also show potential as markers for disease.

The knowledge gained with the ATHERO_MASS project revealed that LDL particles are more complex systems than previously understood, and that changes taking place in LDL during inflammation and disease are multitudinous, involving the lipids, protein and sugars. This corroborated the approach taken during the ATHERO_MASS project, i.e to look at the interactions between components of LDL by mass spectrometry, and shows that LDL is an informative reporting system for cardiovascular health. Methodology was developed that will facilitate further scientific and clinical studies of diagnostic markers, thus providing a powerful tool for the biomedical community. The ATHERO_MASS project has provided new understanding about LDL oxidation in cardiovascular disease that may now be developed and translated for clinical applications, or applied to other research areas from vascular biology to neurosciences, from cell signalling to innate immune system, from inflammation to drug development.

Working on the ATHERO_MASS project has expanded the experience of Dr Reis from chemistry and mass spectrometry into biology and clinical diagnosis, and her work has been recognised by the Hermann Esterbauer Award 2012, presented at the 16th Meeting of the Society for Free Radical Research - International, London 2012.

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