Abstract: P973

Detection of dynamic change in levels of plasma oxidized low density lipoprotein during coronary artery bypass grafting using a natural monoclonal antibody

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Topic(s):
Inflammation and immunity

Citation:
European Heart Journal ( 2016 ) 37 ( Abstract Supplement ), 187-188

Funding Acknowledgements:
National Institute of Health Research Imperial Biomedical Research Centre

Background: We used a naturally occurring antibody (mAb LO1) against oxidised low density lipoprotein (oxLDL) to develop a new assay that measures levels of oxLDL in plasma. We characterised the assay with enzyme-linked immunosorbent assay (ELISA), Western Blotting as well as immune precipitation and mass spectrometry then the tested the ability of the assay to detect dynamic changes during conventional coronary artery bypass grafting (CABG).

Purpose: To develop a new assay to measure levels of oxLDL in plasma and demonstrate its ability to reflect dynamic oxidative stress.

Methods: We developed a sensitive and specific ELISA capture for oxLDL using a well characterised anti MDA-LDL antibody isolated in our laboratory (termed mAb LO1). We then characterised the assay using immunoprecipitation of patient plasma, western blotting, mass spectrometry as well as ELISA. We tested the assay on plasma samples from ten patients (age 65.1±8.2 years) undergoing conventional CABG, taken at four different time points: baseline, 60, 120, and 300 minutes.

Results: We established that mAb LO1 can immunoprecipitate malonaldehyde modified LDL (MDA-LDL) prepared in the laboratory, but not unmanipulated LDL, as detected by western blotting for Apolipoprotein B (ApoB). We then used this approach to determine whether mAb LO1 recognises antigen in human plasma, and were able to isolate a clear band in the ~200kD range. This was confirmed by mass spectrometry to be ApoB (77% peptide match), and to contain oxidation products. We then optimised a capture ELISA using immobilised mAb LO1 and HRP or biotin tagged polyclonal anti-ApoB for detection. We demonstrated variability amongst patient, stability on freeze, thaw cycles as well as ability dynamically detect changes in oxLDL content in plasma subjected to bench-top oxidation. Furthermore, we demonstrated lack of correlation of the new assay with an established Lp(a) assay in a cohort of patients undergoing coronary angiography.

In patients undergoing CABG using conventional bypass studied longitudinally over 5 hours, we found that despite the reduction in ApoB levels between baseline and 60 minutes, likely secondary to haemodilution on bypass, there was an increase in MDA-LDL levels with a significant increase in the MDA-LDL/ApoB ratio at 60 minutes (% change 33.0 ± SD185.2, p<0.05) followed by significant reduction and renormalization of the ratio during recovery by the 120 minute time point with little difference from baseline (-14.0% ± 32.6, p>0.05).

Conclusion: We characterised a novel assay that utilises mAb LO1 to recognised oxidised LDL in plasma and can be used in a capture ELISA to detect temporal changes in patients undergoing CABG surgery. This assay may have several applications including determining oxidative injury and response to treatment.
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