Molecular imaging of experimental atherosclerosis using anti-malondialdehyde-modified low-density lipoprotein humanised antibody fragment targeted nanoparticles

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Introduction: Oxidative modification of low-density lipoprotein (LDL), for example by malondialdehyde (MDA) adduction with subsequent uptake by macrophages to form foam cells and later the plaque necrotic core, is a key initiating event in atherogenesis. Accordingly, a larger lipid necrotic core is a key plaque vulnerability factor, predisposing plaques to rupture and subsequent thrombosis and development of an acute coronary syndrome. Thus, MDA-LDL is an attractive focus for the molecular targeting of atherosclerotic plaques.

Purpose: To develop antibody fragment-targeted nanoparticles that can be utilised for both the molecular imaging and therapeutics of vulnerable atherosclerotic plaques.

Methods: LO1 is an IgG3k natural monoclonal murine antibody that reacts with MDA-LDL. Humanised LO1Fab fragments have been engineered to reduce immunogenicity and improve lesion penetration. These humanised LO1Fab fragments were used to functionalise fluorescent poly(lactic-co-glycolic acid) (PLGA) - polyethylene glycol (PEG) nanoparticles. Nanoparticle in vitro function was assessed, prior to fluorescence molecular tomography (FMT) co-registered with micro-CT, four-hours after iv injection in atherosclerotic LDL-receptor−/− mice fed a high-fat diet for 40-weeks.

Results: Humanised LO1Fab fragment conjugated fluorescent PLGA-PEG nanoparticles were formulated with 210nm size and polydispersity index (variability of nanoparticle size around the average) of <0.2. Antibody conjugation efficiency was 30%. In vitro function was confirmed on ELISA versus the blank untargeted nanoparticles with MDA-LDL on solid phase, detecting nanoparticle presence via the conjugated LO1Fab, PEG corona or fluorescence. Fluorescence microscopy on stained aortic root cryosections from atherosclerotic mice confirmed binding to fatty lesions. Construct in vivo in half-life was 90-minutes for both the targeted and untargeted nanoparticles in a two-phase model in LDL-receptor−/− mice, based on fluorescence analysis of serial tail vein blood samples. There was greater uptake in the region-of-interest (heart and aortic arch vessels) in mice injected with LO1Fab-conjugated nanoparticles versus untargeted nanoparticles (mean ± standard deviation) (64.7±22.9 versus 25.2±26.5 pmol of Cy5; n=7; p=0.02). Ex vivo analysis fluorescence reflectance imaging and quantitative FMT of the extracted aortae confirmed these findings (1.0±0.3 versus 0.5±0.2 pmol of Cy5; n=7; p=0.002; Figure 1).

Conclusions: Humanised antibody Fab fragment fluorescent nanoparticles have been developed that successfully target MDA-LDL and localise to atherosclerotic plaques in murine experimental atherosclerosis. These targeted nanoparticles have the potential to amplify fluorescent signal for imaging and carry a therapeutic cargo for targeted drug delivery direct to atherosclerotic plaques.