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FINAL REPORT

Project / Program Title		Innovative methods for detection and stabilisation of unstable atherosclerotic plaques
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PROJECT SUMMARY

Strokes and heart attacks are typically caused by abrupt rupture of unstable atherosclerotic plaques in the arteries. Ability to differentiate these rupture-prone unstable plaques from stable plaques is still limited. Advances towards understanding and prevention of plaque rupture have been hampered by lack of animal models that reflect plaque instability/rupture as seen in humans. We use a unique mouse model of plaque instability/rupture that closely reflects human pathology which was established in our laboratory. Based on this novel animal model and several unique biotechnological tools, we aim to systematically develop and test: (1) imaging technologies for the identification of unstable, rupture-prone plaques and (2) innovative plaque-stabilising therapies. Detecting and treating plaque instability will prevent plaque rupture and its associated complications, such as strokes and heart attacks.

PROJECT AIMS / OBJECTIVES

Our specific aims are:

(1) In vivo identification of unstable plaques by targeting activated platelets as markers of plaque instability using Positron emission tomography (PET)/computed tomography (CT)

(2) Stabilisation of rupture-prone atherosclerotic plaques via an innovative, novel approach (Site-directed delivery of CD39 targeting activated platelets)

Aim 1:

Rationale for platelet targeting: Activated platelets are involved in plaque instability via three potential mechanisms: (1) adhering to inflamed endothelium; (2) accumulating within intraplaque haemorrhages; and (3) forming thrombi on exposure to prothrombotic materials during plaque rupture. Using GFP-labelled platelets, platelets have been shown to be recruited selectively to the

areas of plaque instability. Thus, platelet targeting may offer a long-sought method for detecting unstable plaques, as well as the development of a novel pharmacological strategy towards plaque stabilisation.

Single-chain antibodies (scFv): These are small antibody fragments that can penetrate deeply into tissues. Our lab has extensive experience with scFv technology. We will focus on scFv to glycoprotein GPIIb/IIIa, which specifically binds to the activated platelet integrin receptor GPIIb/IIIa. On platelet activation, GPIIb/IIIa undergoes a conformational change and exposes epitopes that are specific for activated platelets. This, together with GPIIb/IIIa's abundance, makes this target ideally suited for molecular imaging and therapeutic targeting with absolute selectivity for activated platelets.

Positron emission tomography (PET)/computed tomography (CT): These platelet-targeted scFvGPIIb/IIIa will be radio-labelled with Copper-64(64Cu) as 64Cu, with a half-life of 12.7 hours, is an emerging isotope of choice for preclinical and clinical imaging. The conjugate will be injected intravenously (via tail vein) into ApoE-/- mice subjected to tandem stenosis surgery. Mediso Nano- PET/CT small-animal scanner will be used to perform this platelet targeted molecular imaging to investigate the accumulation of radiotracer at the sites of vulnerable plaques as a proof of concept. PET/CT is ideal for targeted molecular imaging with its excellent signal sensitivity and specificity. Pilot experiments suggest that activated platelet targeting of a 64Cu radiotracer in PET represents a long-sought approach for the identification of unstable, rupture-prone plaques.

Aim 2:

Rationale for CD39 as an innovative anti-platelet and anti-inflammatory drug: Adenosine tri- and di- phosphate (ATP and ADP), released either actively by cells or passively during cell lysis, are strong proinflammatory and prothrombotic agents. In contrast, their final catabolic product, adenosine, is a strong anti-inflammatory and anti-platelet agent. The enzyme CD39 degrades ATP to ADP as well as ADP to AMP (adenosine monophosphate), and the enzyme CD73 degrades AMP to adenosine. Inactivation of CD73 promotes atherosclerosis and atherosclerotic vessels in ApoE-/- mice show significantly reduced CD39 expression. Interestingly, regulatory T-cells (Tregs) strongly express CD39, which is believed to contribute to their atheroprotective effect. In human coronary arterectomy samples, plaque stability is notably associated with an increase in CD39 expression, suggesting CD39 plays an important role in the prevention of plaque rupture and thus MI.

Hence, the therapeutic application of CD39 offers strong anti-inflammatory and anti-platelet potential.

CD39 specifically targeted to activated platelets: Inhibition of platelet ADP receptor P2Y12 by clopidogrel, prasugrel and ticagrelor has been highly successful in atherosclerosis. However, these drugs are associated with an increased risk of bleeding complications. Similarly, administration of effective doses of CD39 also results in an increased bleeding tendency. But targeting CD39 to epitopes enriched at unstable plaques (e.g. activated platelets) will allow its anti-inflammatory action to be selectively directed to vulnerable plaques without systemic side-effects. We will use genetically fused CD39 to scFvGPIIb/IIIa, which will selectively target anti-thrombotic CD39 activity to activated platelets and clots. However, based on its low systemic concentration, this CD39 fusion protein did not result in bleeding time prolongation. Using TS mouse model, we will investigate the potential plaque stabilising effect of platelet-targeted CD39 in vulnerable plaques.

Brief description of tandem stenosis (TS) mouse model

In ApoE-/- mice at 12 weeks of age, after 6 weeks on a high-fat diet, a tandem stenosis is induced by two sutures on the right common carotid artery. This results in the development of unstable plaques, which reflect the characteristics of plaque instability/rupture as seen in human, such as thin and/or ruptured fibrous cap, intraplaque haemorrhages, large necrotic cores, plaque inflammation, positive vascular remodelling, neovascularisation and intravascular thrombus

formation. This model provides unique samples of unstable plaques in TS segment, stable plaques in controls and aortic arch and normal healthy arterial tissues.

SIGNIFICANCE AND OUTCOMES

Despite considerable progress in imaging technologies, in clinical practice we are not yet able to reliably identify atherosclerotic plaques that are prone to rupture. As there is currently no clinical tool available to identify unstable plaques, myocardial infarct (MI) and stroke can be very difficult to predict. As a consequence, many patients suffer sudden cardiac death or a fatal stroke. Patients who make it to hospital are most often too late to fully salvage the ischaemic brain or myocardium and develop long-term disabilities and/or heart failure. Overall, the mortality and morbidity resulting from strokes/MI present a significant burden on our health care systems.

However, the biology of plaque rupture provides a major opportunity to substantially change the scope of how we approach atherosclerosis. Rupture-prone plaques, so-called vulnerable or unstable plaques, have specific characteristics that can be harnessed for molecular diagnostic imaging and targeted therapy (activated platelets localised in vulnerable plaques in our studies). This application focuses on innovative technologies for detection and preventive treatment of vulnerable plaques as one of the most challenging, but also most rewarding, aims in cardiovascular medicine.

Although currently proposed experiments are designed for preclinical/animal studies, the single chain antibody constructs are based on human antibodies and these should be transferable to human studies if proof of concept works, hence it has potential translational values.

PUBLICATIONS / PRESENTATIONS

Still need a couple more years to complete the project in full. No publications to date.