

IMAGING VULNERABLE ATHEROSCLEROTIC PLAQUES WITH RADIOLABELLED SINGLE CHAIN ANTIBODIES

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Background

The chronic inflammatory disease, atherosclerosis is a major underlying cause of ischaemic heart disease. Rupture of unstable atherosclerotic lesions can lead to heart attack and stroke. These are the most common cause of sudden death in Australia. Activated platelets play a major role in the development of lesions, both in early stages and in formation of clots after plaque rupture. We previously generated a single-chain antibody (scFv) that specifically binds to the abundant platelet surface receptor glycoprotein integrin IIb/IIIa in its activated, ligand-bound form (LIBS). The purpose of this study is to label LIBSscFv with F18 to investigate its potential as a PET radiotracer to detect activated platelets.

Methods

Both LIBS-scFv and control-scFv were conjugated to N-succinimidyl 4-[¹⁸F]fluorobenzoate (S[¹⁸F]FB), a radioactive NHS ester compound. ScFvs were reacted with 6.25 mCi of SFB for XY min. The radiolabeled antibody was characterised using SDS gels and western blotting. Binding of the scFv to activated-platelets in vitro and in vivo was also assessed. After radiolabeling, binding was examined by flow cytometry and compared to unlabeled scFv. In vitro platelet rich clots were formed from blood collected from healthy human volunteers and incubated with different concentrations of the radiolabeled scFv. The amount of bound radioactivity was measured using both the PET scanner and directly measured using a gamma counter. In vivo experiments were performed in a mouse model of acute thrombosis where a platelet rich clot is formed in the carotid artery using FeCl₃. Afterwards the radiolabeled scFv was injected intravenously and a 1-hour dynamic PET scan was performed using a Mosaic small animal PET scanner. Both injured and non-injured vessels along with all

the major organs were collected for biodistribution and histological studies after scanning.

Result

Radioactivity measurements show an increase in bound radioactivity in clots incubated with LIBS-scFv compared to control-scFv. After incubation with radiolabeled scFv-LIBS clots retained significantly greater ($p = 0.04$, $n=9$) amounts of radioactivity compared to clots incubated with radiolabeled scFvcontrol. Clots incubated with LIBS-scFv retained 3.5% of activity (decay corrected) compared to clots incubated with the non-binding scFv which retained 1.3% of radioactivity. Increasing radioactivity in urine demonstrates fast renal clearance of the labelled scFv. FeCl₃ injured vessels showed high radioactivity uptake compared to non-injured vessel, shown by gamma counter measurements as well as image analysis of the PET scan images. Only minimal uptake of the radiolabeled control scFv was seen in injured vessels. Presence of activated-platelets was determined by histology subsequently.

Conclusion

High specific uptake of radiolabeled LIBS-scFv in the mouse model shows indicates that this novel radiotracer is suitable for detecting activated platelets associated with rupture-prone lesions. Radiolabeled LIBS-scFv can potentially identify patients with serious atherosclerotic disease much earlier than currently possible, thereby allowing early initiation of medical treatment to stabilise lesions. This has significant implications for disease management and prevention of suffering caused by myocardial infarction and stroke.