YIA applicant

54

A leukocyte ligand of vascular adhesion protein-1 as an imaging tool in PET

Autio A. [1], Saanijoki T. [1], Sipilä H. [1], Jalkanen S. [2], Roivainen A. [3].

[1] Turku PET Centre,
[2] MediCity Research Laboratory, National Public Health Institute,
[3] Turku PET Centre, Turku Centre for Disease Modeling.

akauti@utu.fi

Introduction: Vascular adhesion protein-1 (VAP-1) is both an endothelial glycoprotein and a semicarbazide-sensitive amine oxidase (SSAO) enzyme playing a critical role in leukocyte trafficking to the sites of inflammation. Although VAP-1 was identified more than 15 years ago, the leukocyte ligand has remained unknown until very recently. Last year it was shown that Siglec-10 (sialic acid-binding immunoglobulin-like lectin) expressed on a subpopulation of lymphocytes can bind to VAP-1 and serve as its substrate [1]. According to phage display screening and structural modeling also Siglec-9 expressed on granulocytes and monocytes interacts with VAP-1. In this study, we investigated a Siglec-9 peptide as a potential imaging tool in positron emission tomography (PET).

Methods: A cyclic peptide binding to recombinant human VAP-1 was conjugated with DOTA-chelator through PEG-linker and 68Ga-labeled for PET studies as previously described [2]. The interaction between VAP-1 and 68Ga-Siglec-9 peptide was evaluated in vitro in human plasma samples possessing different SSAO levels. The VAP-1 specificity was further tested with competition assay in mice bearing melanoma xenografts by PET imaging and autoradiography. *In vivo* imaging of inflammation was examined in a rat model. All *in vivo* studies were confirmed by ex vivo measurements.

Results: The Siglec-9 peptide binding to the enzymatic groove of VAP-1 could specifically detect inflammation in rat and tumor in mouse. Competition experiments with excess of unlabeled Siglec-9 peptide revealed 3-fold lower tumor uptake in mice. According to autoradiography of the tumor cryosections, the radioactivity co-localized notably with VAP-1 as demonstrated by immunohistochemistry ($P<0.0001$). In a rat model, the Inflammation-to-muscle ratio of 68Ga-Siglec-9 peptide was 5.9±2.3. Moreover, in radio-HPLC analyses, the amount of intact 68Ga-Siglec-9 peptide in human plasma was significantly different between low and high levels of SSAO activity ($P=0.0007$).

Conclusions: Our results show that the Siglec-9 peptide detects VAP-1 in inflammation and tumor vasculature in animal models and it may also have potential in imaging of these diseases in patients.

Acknowledgement: The study was conducted within the Finnish CoE in Molecular Imaging in Cardiovascular and Metabolic Research supported by the Academy of Finland, University of Turku, Turku University Hospital and Åbo Akademi University. Anu Autio is a PhD student supported by the Drug Discovery Graduate School.

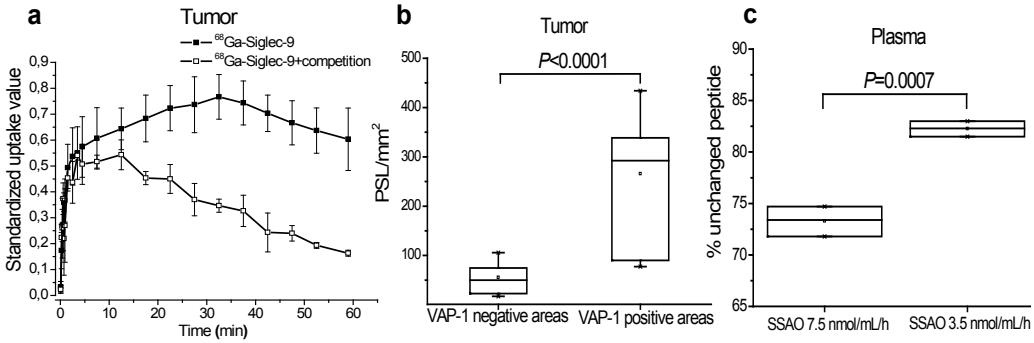

Fig1: Uptake of 68Ga-Siglec-9 peptide in melanoma xenografts in mice is specific and correlates with VAP-1 expression. (a) PET study (b) Autoradiography study (c) HPLC study.

References:

1. Kivi E et al; Blood. 114:5385–5392 (2009)
2. Ujula T et al; Nucl Med Biol. 36:631-641 (2009)