Noninvasive In Vivo High-Resolution MRI of vessels affected by transplant rejection in mice: As good as histopathological analysis?

A. Hess[1], J. Gehardt[2], L. Budinsky[3], U. Reulbach[4], and S. M. Ensminger[2]

[1]Pharmacological Imaging, I. f. Pharmacology, Erlangen, NA, Germany, [2]Cardiac Surgery, University of Erlangen Nuremberg, [3]I. f. Pharmacology, [4]Psychiatry and Psychotherapy, University of Erlangen Nuremberg

Introduction: Major limitation of the investigation of transplant vasculopathy in mice is, that it's difficult to monitor the progression and potential response to therapy in vivo. The aim of this study was to show that the detection of the residual lumina by MR angiographic imaging is comparable with those achieved by histopathological analysis.

Methods: ***Animals***

CBA.J ($H2^k$) and C57BL/6 ($H2^b$) mice were used as recipients and donors of the aortic allografts or isografts. All mice ranged in age from 8 to 12 weeks at the time of experimental use under specific pathogen-free conditions and treated according to institutional and state guidelines.

Treatment and Protocol: The mice underwent no pharmacological intervention especially no immunosuppressive or immunomodulatory medication to prevent transplant rejection. Experimental animals were divided into two groups each animal received a fully mismatched aortic transplant on day 0. Group 1 underwent MRI measurement and histopathological analysis on day 35 and group 2 on day 65 after transplantation.

Abdominal Aortic Transplantation

Allogeneic and syngeneic aortic transplantations were performed with a modified technique initially described by Koulak et. al[1]. In brief, the donor thoracic aorta was isolated, resected, and transfered to the recipient animal. The recipient aorta was clamped and then transsected with sharp microvascular scissors. A proximal end-to-end anastomosis was performed. The aortic graft was then repositioned, and the anastomosis was continued with single interrupted sutures.

MRI methods

MRI was performed on a 4.7 T BRUKER Biospec scanner with a free bore of 40 cm equipped with quadrature mouse volume coil enabled homogenous excitation used as a receiver and transmitter coil. After proper positioning the scanning procedure started with the acquisition of series of 10 coronal slices with Flash sequence, FOV 5cmx5cm , slice thickness 0.93 mm, matrix 256x256, TR = 80 ms, TE =2.8 ms, flip angle =30 degree, total measurement time 3 min. Angiography images were at first acquired using non triggered 3D inflow technique (3D TOF: excited slab dimensions were 35 x 35 x 35 mm, measured matrix dimensions were 256x256x128, TR = 15 ms, TE =2.5 ms, 2 averages, flip angle 30 degrees, total measurement time 16 min). Second angiography measurement was done by using 3D Phase Contrast Angiography (PCA) technique (excited slab dimensions were 35 x 35 x 35 mm , measured matrix dimensions were 224x224x96, TR = 15 ms, TE =2.5 ms, 2 averages, flip angle 30 degree, maximal velocity = 40 cm/s, flow , Hadamard flow encoding scheme, total measurement time 45 min). The scanning procedure continued with the acquisition of T2 weighted spin echo 2D anatomical images (slice thickness 0.5 mm, 32 axial slices, field of view 30 x 30 mm, matrix 256 x 256, TR = 3850 ms, TEef = 56 ms, 8 averages, total measurement time 20 min) using a rapid acquisition relaxation enhanced sequence (RARE) with rare factor equal 8.

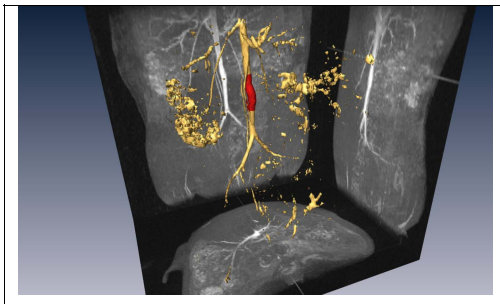
Analysis of the Aortic graft: Aortic grafts were harvested after 35 days group 1 and day 65 in group 2. The grafts were perfused with saline, frozen in OCT medium and cutted to 12 7-µm thick cross-sections and placed on microscopic slide and stained with Elastin-van Gieson.

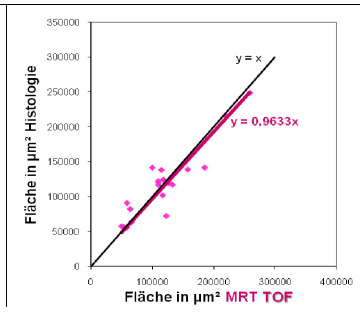
Morphometry:5 histological sections from each graft were analyzed by two indepent examiners at an original magnification of x200 using a conventional light microscope. Photomicrographs of the histopathological sections were impoted digitally into ANAlysis Image Analysis software (Olympus, Hamburg, Germany) and subsequently computer morphometry was performed using ImageJ. Different diameters, total area with the lumen and a quotient for the thickness of the intima (Q_{int} in %) was calculated. The MR angiograms were segmented and reconstructed by custom made software and visualized with Amira (Visage Imaging GmbH).

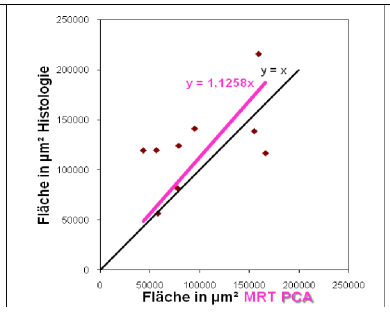
Statistical Analysis: Results were given as the mean per graft both in MRI analysis and in histology. The histological data were analyzed by using a 2-tailed paired Student t-test. The correlation between the intima proliferation caused lumen narrowing and the appropriate MRI flow data was calculated by linear regression analysis. In addition, for comparison of the methods of histological analysis and MR imaging the Bland-Altman Plot was used.

Results & Discussion: 3D angiographic imaging and reconstruction of abdominal implanted aortic vessels is possible in the anesthetized mouse. There was eminent correlation between MR and histopathology measurement in determination of residual lumina, especially using the 3D TOF MRI methode (r=0,93±0,04 vs r=0,65±0,37 PCA method). Clearly, there is an auspicious role for noninvasive imaging in tracking the implications of transplant vasculopathy in the form of lumen narrowing in murine models. By further improvement of the resolution or development of target specific contrast agents MR imaging could possibly replace histopathology as the assessing instrument.

Acknowledgements: Erlanger IZKF Z2







Left side: Reconstructed 3D TOF MRA with the implanted vessel colored in red. Middle side: Correlation plot of the area size obtained by TOF to the area of histology, right same as middle but for PCA versus histology.