In vivo molecular MRI using an elastin-binding contrast agent investigating carotid artery injury in mice

M. R. Makowski[1], U. Sausbier[2], Y. Liao[2], M. Schwaiger[1], W. Neuhuber[2], P. Ruth[2], M. Sausbier[2], and R. M. Botnar[1]

[1]Nuclear Medicine, Technical University, Munich, Bavaria, Germany, [2]LS Pharmakology and Toxikology, Pharmacology, Tuebingen, Germany

Introduction

After vascular injury, extracellular matrix (ECM) turnover and smooth muscle cell (SMC) proliferation are thought to play an essential role in vessel wall repair. The cysteine-rich protein 2 (CRP2), was recently identified as novel and specific molecular effector of the NO/cGMP/cGMP-dependent protein kinase I (PKG) signaling in vascular smooth muscle. We established a CRP2-deficient mouse line by targeted deletion of the exons 2-7 in the CRP2 gene to investigate whether CRP2 contributes to the postulated pro-proliferative/proatherogenic effect of PKG. Using an up-graded MR scanner and a novel ECM-specific MR contrast agent it should be feasible to assess changes in remodeling of the injured carotid wall in mice.

Purpose

In this study we investigated whether the use of an elastin-binding contrast agent would allow the detection of vascular remodeling in a mouse model of carotid artery injury and whether it would facilitate the detection of an impaired ECM formation in CRP2-/- mice.

Methods

A carotid artery injury model was performed in 8 CRP2-deficient (CRP2-/-) and 8 wild type mice (WT) the left served as control the, while right carotis was damaged., MRI of the carotid vessel walls was performed two month after the surgical intervention in a 1.5T Philips Achieva clinical MR scanner using a dedicated software package (R1.2.2), a single loop small animal coil (∅ = 23mm) and a clinical gradient system (30mT/m, 150mT/m/ms). For visualization of the injured vessel segment and planning of the vessel wall scans time-of-flight (TOF) angiography of the carotid arteries was performed. Imaging parameters included TR=43, TE=8.1, flip angel=60°, spatial resolution=0.2x0.2x0.5 mm. For assessment of ECM remodeling, an inversion recovery (IR) vessel wall sequence was performed approximately 45-60 minutes post injection of 0.1mmol/kg of CP1052 (Bristol Myers Squibb), a novel elastin-binding Gd^{2+}-based contrast agent. Imaging parameters included TR=44, TE=11, flip angel=30°, spatial resolution=0.1x0.1x0.5 mm, 22 lines per RR interval, inversion time approx. 250ms. Signal-to-noise ratio (SNR) and contrast-to-noise (CNR) of the injured vessel wall was determined by manual segmentation of the visually apparent signal of the contrast agent below the carotid bifurcation. To verify our MR data, we performed a histological elastica staining in 10µm-whole-neck paraffin slices (Elastica-Van Gieson method).

Results

All animals completed the MR examination without any adverse events. SNR and CNR of the injured right compared to the non injured left carotid artery was significantly increased ($p<0.001$) both in wild type (Figure 1A) and in CRP2-/- (Figure 1B) mice (SNR: 9.4±1.6 vs. 6.4±2.1; CNR: 7.9±1.8 vs. 4.9±1.7). More interestingly, SNR and CNR of the injured right carotid artery of CRP2-/- mice was significantly decreased when compared to control animals ($p<0.05$) (Figure 3). No significant difference was found between non-injured carotid arteries of both genotypes. In both genotypes, the carotid injury *per se* was confirmed with Elastica Van Gieson staining (Figure 2). Preliminary evaluation (semi-quantitative analysis currently under investigation) of the carotid wall thickening seem to confirm our MRI findings and suggests that targeted deletion of CRP2 in mice might lead to a reduced vessel wall thickening and therefore to a reduced restenosis after vascular injury.

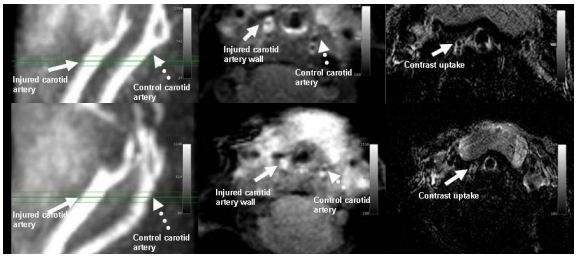

Figure 1: Increased CP1052 signal in the injured right carotid artery of CRP2-/- mice compared to controls.

Conclusions

In this study, we demonstrate the successful non-invasive assessment of alterations in the vessel wall after vascular injury in a mouse model of impaired smooth muscle cell proliferation and ECM formation using molecular MRI. Molecular alterations in the injured and non-injured vessel wall, as well as between wild type and CRP2-/- mice with regard to elastin formation after vascular injury can be differentiated using CP1052.

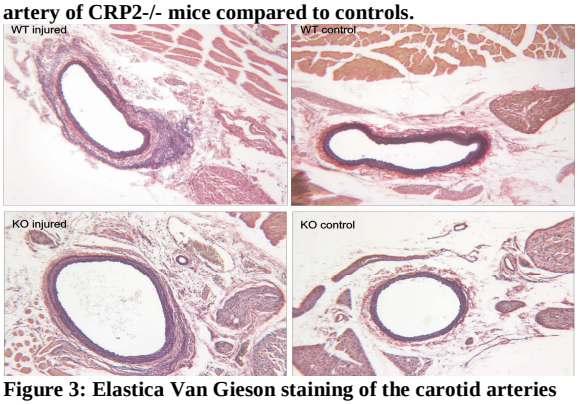

Figure 3: Elastica Van Gieson staining of the carotid arteries of WT, CRP2-/- mice (KO).

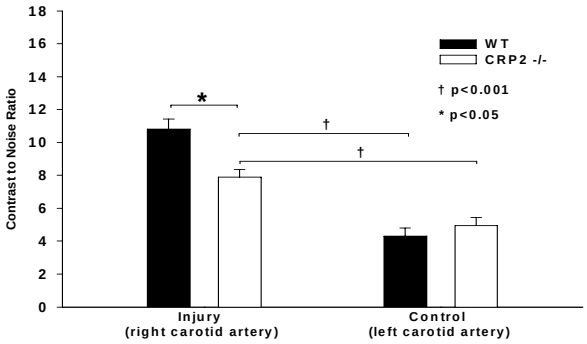

Figure 2: Significant increase of SNR and CNR of the injured right carotid artery of CRP2-/- mice compared to control animals. SNR (12.9±2.2 vs. 9.4±1.6), CNR (10.8±1.8 vs. 7.9±1.8).