Imaging vasulcar injury using a novel gadolinium-based contrast agent

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Introduction: The vasculature is one of the most promising targets for a site- and function-specific MRI strategy, as the diagnosis of vascular disease in its early stages is essential to a successful treatment intervention. Endothelial regeneration following percutaneous coronary artery interventions is emerging as a potentially crucial factor preventing re-stenosis and late thrombosis. Endothelial damage is one of the essential early steps in progression of vascular diseases. Therefore, development of contrast agents specific for endothelial damage would be beneficial not only for diagnosis of vascular disease in its early stage, but also for monitoring vascular regeneration. There is currently a limited availability for contrast agents detecting vascular endothelial damage, especially the loss of endothelium. Most of the agents are designed using biomolecules such as antibodies for integrin, ICAM-1 and fibrin, for imaging conditions such as thrombosis, atherosclerotic lesions and activated endothelium. We have previously used a rat vascular injury model for assessing vascular regeneration and involvement of vascular progenitor cells in the healing process. Here we report the use of an endothelial-lesion specific contrast agent, evans-blue chelated gadolinium (EB-DTPA-Gd) for non-invasive MR imaging of vascular damage. Furthermore, we have investigated the localisation of the agent by using a combination of T2-weighting and ex vivo high-resolution imaging at 9.4T.

Methods: *Rat (Spraque-Dawley) carotid artery balloon injury model;* 2F embolectomy catheter was introduced *in vivo* via the external carotid artery (CA) to the common CA, inflated with air and rotated while retracting in order to remove the endothelium. *EB-DTPA-Gd & MRI;* 67.96 µmol of EB-DTPA-Gd/ rat (Yoshiki Katayama, Kuyshu Univ. Japan) was slowly injected in the tail-vein immediately after balloon angioplasty. The CAs were excised 20 min later, cleaned from surrounding tissue and gently inflated with PBS (Fig 1). The arteries were placed in 1.5mL eppendorf tubes filled with PBS, and the phantoms were scanned using a 9.4T Varian (VNMRS) scanner. T1-weighted spin-echo axial and longitudinal MR images of the left and right carotid arteries were collected (axial: FOV 20x10mm, 512x256 matrix, 1mm slc, TR=500, TE=11, 20av. long: FOV 40x20mm, 512x256 matrix, 0.5mm slc, TR=400, TE=11, 20av). T1/T2-weighted images were also acquired with a fast spin-echo sequence (FOV 20x10mm, 512x256 matrix, 0.8mm slc, TR= 450ms and 650ms, effectiveTE= 10ms and 18ms, 50av).

Results: EB-DTPA-Gd accumulation and the corresponding increase in SI clearly distinguished the left (injured) arteries from the right (control) (Fig 2). Accumulation of EB-DTPA-Gd was found throughout the denuded artery. Increasing T2 weighting caused signal loss in the arterial wall and confirmed that the agent was bound on the luminal side of the artery (Fig 3). No acute toxic effects were seen following delivery of EB-DTPA-Gd, and renal clearance of unbound agent was noted within 20mins of administration.

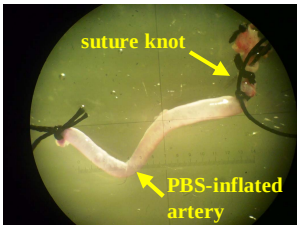

Figure 1: *Ex vivo* artery prior to placement in eppendorf tube.

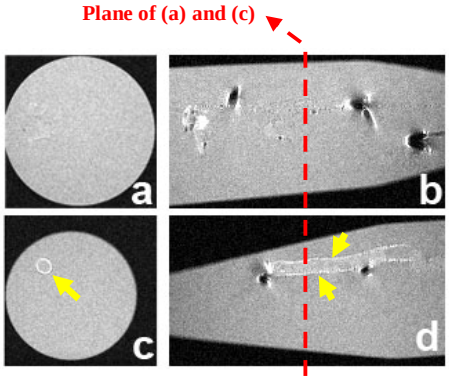

Figure 2: EB-DTPA-Gd and endothelial injury. T1-weighted spin-echo images of eppendorf phantom. Non-injured control arteries (top images, **a** and **b**) and injured arteries (bottom, **c** and **d**) were visualised in the axial (**a**, **c**) and longitudinal (**b**, **d**) directions. The agent caused a clearly visible increase in signal and highlighted the injured artery (yellow arrows).

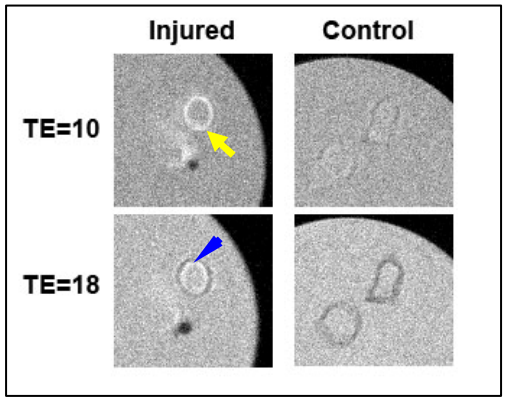

Figure 3: Localisation of EB-DTPA-Gd. At shorter TE (top) the signal from the artery was similar to that of PBS and was enhanced by the presence of Gd3+ in the injured artery (yellow arrow). At longer TE (bottom) there is signal loss in the arterial wall, and the Gd3+ enhancement is restricted to the luminal side of the artery (blue arrow).

Conclusions: Evans Blue is a well established agent to assess perfusion and endothelial damage, and once conjugated to gadolinium offers a novel site-specific imaging tool for vascular damage and endothelial regeneration. Yoshiki Katayama *et al* have previously shown that with the injection of EB-DTPA-Gd *in vivo,* the signal intensity from an ROI including the injured artery was enhanced in comparison to the control artery. Here we have presented a phantom design with which high resolution *ex vivo* images were possible. Evans blue-chelated gadolinium revealed signal enhancement only on de-endothelialised carotid arteries in T1-weighted images. We have also provided evidence of the localisation of the agent solely to the luminal side of the artery. Selective binding of EB-DTPA-Gd to the target regions in vascular injury, even in the presence of serum or the blood stream, offers a potentially useful tool for development of a diagnostic system detecting vascular disease in its early stages. We plan to use this imaging tool for *in vivo* monitoring of vascular damage and regeneration in animal model of vascular damage, following administration of endothelial progenitor cells and / or therapeutic vectors.

Ref: Tatsuhiro Yamamoto, Kenjiro Ikuta et al.Bioorganic & Medicinal Chemistry Letters, 14; 2787-2790, 2004