MR angiography and MR spectroscopy of deoxymyoglobin for efficacy assessment of gene therapy in critical limb ischemia

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Introduction

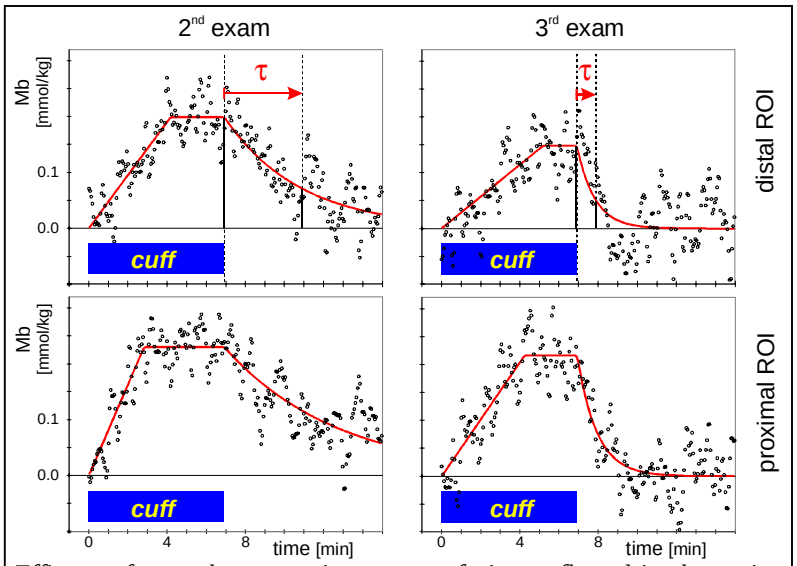
Growth factor-induced neovascularization to promote reperfusion of ischemic tissue is a promising, but clinically still unproven therapeutic approach in patients with chronic critical leg ischemia (CLI)[1,2]. To evaluate the efficacy of therapeutic neovascularization, reliable surrogate endpoints are needed to document changes in perfusion, because ideal clinical endpoints are not available. In this work, the potential of MR angiography to detect collateral vessel growth and the use of ^{1}H-MRS of ischemia-induced deoxy-myoglobin (Mb) to document perfusion changes were evaluated.

Methods

MRA was performed on a Siemens 1.5 T scanner using a 39-cm extremity coil and the following parameters: coronal 3D gradient-echo MRI with: 4.6 ms TR, 1.8 ms TE, 30° flip angle, 190 x 380 x 100 mm FOV, 1.53 x 0.76 x 2 mm raw pixel size, 28.5 s per series, 1 pre- and 2 consecutive post-contrast series, bolus of 30 ml gadodimid (Omniscan™) injected at 3 ml/s, followed by a saline flush. Subtraction MIP images were reconstructed in 15° steps with 4 images documented on film. Follow-up images were reconstructed to reflect baseline orientation. MRAs were reviewed by 3 independent blinded observers using a predefined semiquantitative visual protocol to assess stem vessels, and large and small collaterals. Reproducibility of collateral assessment was evaluated in 7 CLI patients in 2 exams (3 ± 2 days apart).

MRS: Deoxy-Mb was measured by ^{1}H MRS on a GE 1.5 T MR system as described in Ref [3] (selective excitation of deoxy-Mb signal at 78 ppm, 110 ms TR, 2 independent surface coils to examine simultaneously 2 regions of ~50 cm^3 at 7 (distal) and 22 cm (proximal) above the medial malleolus). Effective muscle reperfusion was characterized by the recovery time constant (τ=1/k) found for the exponential disappearance of the deoxy-Mb signal induced by 7 min. of ischemia. Data processing included prior-knowledge fitting, standardization to the tissue water signal, and modeling of the Mb signal to a time course consisting of a linear increase, a plateau and an exponential decay. Reproducibility of ^{1}H MRS was evaluated in 5 CLI patients in 2 independent sequential examinations.

Patients: Collateralization and perfusion were studied in 12 CLI patients undergoing intramuscular pVEGF-C gene therapy (n=4), percutaneous transluminal angioplasty (n=4), exercise training (n=2), or no therapy (n=2). (Single-dose, later followed by multiple-dose *VEGF-C gene therapy* (Vascular Genetics Inc., USA) similar to Ref [2]). Limb ischemia was documented by ankle brachial and great toe pressure; arterial occlusions by an intra-arterial contrast angiogram.



Efficacy of gene therapy to increase perfusion, reflected in shortening of the recovery of the Mb signal, when comparing MRS results obtained before and after multiple dose gene therapy in a single patient

Results

Despite remarkable inter-observer agreement in the reproducibility and treatment studies, the correlation of **MRA** changes with clinical course was poor, since agreement between MRA and clinical course and pressure measurements was 67% and 60%, respectively. Changes were most often observed at the level of small sized collaterals. CV of recovery times from **MRS** between exams was 20-30% at the two locations. τ values depended on anatomic level and extent of arterial occlusion. Changes were temporally related and accorded with clinical course. Agreement between ^{1}H MRS and clinical course and pressure measurements was 93% and 71%, respectively. A single case is given as example: Clinical deterioration after single dose gene therapy was documented by substantial lengthening of recovery times, while follow-up multiple dose gene therapy brought about clinical improvement, which was reflected in drastic shortening of the recovery times (Fig).

Discussion

Results show that MRA and ^{1}H MRS of Mb complement each other in documenting changes in collateralization and tissue perfusion in CLI patients. They thus appear well suited to assess the efficacy of therapeutic angiogenesis in CLI.

References: **1.** Khan T.A. et al. *Gene Ther.* **10**:285 (2003); **2.** Baumgartner I. et al. *Circulation* **97**:1114 (1998); **3.** Kreis R. et al. *Magn.Reson.Med.* **46**:240 (2001)

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