In vivo 3D MR angiography reveals accelerated collateral vessel growth in CD73$^{-/-}$ mice after hindlimb ischemia

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Introduction

Adenosine, the dephosphorylation product of ATP, can be formed extracellularly and is part of the adenine nucleotide cascade involving CD39 and CD73 (ecto-5´-nucleotidase). Adenosine acts *via* specific adenosine receptors and plays an important role in the regulation of vascular tone and inflammatory processes. Furthermore, adenosine has been reported to be a potent stimulator of angiogenesis. However, its role in arteriogenesis is not known. To address this issue we induced hindlimb ischemia in a mouse mutant lacking CD73. The phenotype of these mice was previously shown to be associated with enhanced monocyte adhesion to the endothelium, considered to be an important initial trigger for arteriogenesis[1,2]. Collateral vessel growth was assessed by 3D MR angiography (MRA) and metabolic restoration by *in vivo* ^{31}P MR spectroscopy.

Methods

Experiments were performed at a vertical Bruker DRX Wide Bore NMR spectrometer operating at frequencies of 400.13 MHz for ^{1}H and 161.97 MHz for ^{31}P measurements using a Bruker Microimaging unit (Micro 2.5) equipped with an actively shielded 40-mm gradient set (1 T/m maximum gradient strength, 110 µs rise time at 100% gradient switching). MR angiograms were acquired with a 30-mm ^{1}H saw resonator, while ^{31}P MR spectra were recorded with a dual-tuned ^{1}H/^{31}P 10-mm tilt resonator (acquisition time, 8 min). Hindlimb ischemia was induced by ligation of the femoral artery distal to the branching of the popliteal artery. For serial comparison of blood flow recovery and direct visualization of newly developed collateral vessels we established a high resolution 3D MRA protocol. A 3D gradient echo flow-compensated sequence was used (FOV 2.56×2.56×1.28 cm^3, Matrix 256×256×128, flip angle 28°, TE 4.9 ms, TR 24 ms, 3 averages). The FOV of 128 mm in z-direction was covered by three overlapping slabs of 64 mm (total acquisition time 3×18 min). Datasets were subsequently 'sticked' together, segmented, and quantified with the self-developed image processing software ECCET for 3D MIP reconstruction.

Results and Discussion

In Fig. 1A the femoral vessel system of a mouse one week after ligation of the left femoral artery is displayed, demonstrating the extent of arteriogenesis (green). The typical corkscrew-like structure of the collateral vessels can be clearly recognized. To account for differences in animal size and vessel geometry, newly developed vessels within the ischemic (right) side were normalized by quantification of an anatomically defined artery segment on the contralateral (left) side. For this segment – between the proximal end of the femoral artery and the branching into the popliteal artery – we determined an average length of 8.1±0.2 mm and a volume of 653±134 nL (n=6). Collateral flow was detected as early as 3 d after ligation reaching a transient maximum 7 d after occlusion. At this time the extent of collateral formation in the ischemic area was substantially higher in CD73$^{-/-}$ mice (+70% vs. wild-type (WT), $p<0.05$, n=7; Fig. 1B).

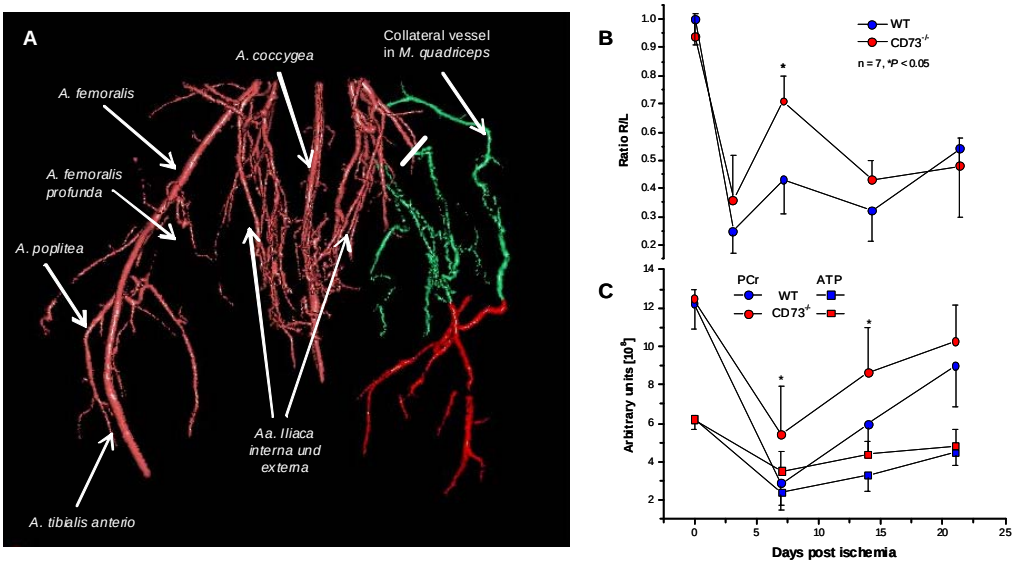

Fig. 1: (A) MRA (MIP projection) from the mouse hindlimb 7 d after ligation of the femoral artery. Newly developed collateral vessels are coloured in green. Time course of arteriogenesis (B) and metabolic recovery (C) in wild-type and CD73$^{-/-}$ mice after hindlimb ischemia (PCr, phosphocreatine; ATP, adenosine triphospate).

The improved blood supply was also reflected by the faster recovery of hindlimb muscle energetics in the mutant compared with WT controls as assessed by *in vivo* ^{31}P MR spectroscopy (Fig. 1C). Histology confirmed the enhanced development of collaterals in CD73$^{-/-}$ mice.

In conclusion, our protocol allows the quantitative assessment of growing collateral vessels in mice. We found that lack of CD73-derived adenosine promotes arteriogenesis.

References

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[2] Heil M et al, *Curr Pharm Biotechnol* 8: 35-42 (2007).