DCE-MRI reveals functional changes in murine kidneys after warm ischemia-reperfusion injury

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Introduction
Kidney transplantation is the preferred treatment for patients with end-stage renal disease. Although the number of available kidneys from brain dead donors is insufficient, the donor pool can be significantly extended by including non-heart-beating donors (NHBD), i.e. patients who die of cardiac arrest. However, these kidneys are additionally damaged by an approximately 45 minute period of warm ischemia between cardiac arrest and cooling of the kidney. This results in a relatively high incidence of primary non-function and delayed graft function of NHBD kidneys1. To improve the clinical outcome of these kidneys, novel therapies are being developed that limit ischemia-reperfusion injury2. To fully assess their therapeutic efficacy at an early stage, new imaging methods are required that allow detection of renal function in a non-invasive and longitudinal manner in both pre-clinical and clinical setups. Here, we describe the application of Dynamic Contrast Enhanced MRI (DCE-MRI) using the separable compartment model3 in combination with a reference region input function4, to assess renal function in a mouse model of ischemia-reperfusion injury in NHBD kidneys.

Methods
In vivo MRI. Male Swiss mice (n=9) were anesthetized with 2% isoflurane in medical air. As a model of warm ischemia-reperfusion injury, the left renal pedicle was occluded for 45 minutes using a small surgical clamp. The right kidney served as internal control. After 24 hours of reperfusion, mice were subjected to the MRI examination on a 7 T Bruker Biospec 70/30 USR. T1-weighted anatomical images with TR 3000 ms and TE 40 ms were recorded to allow correct delineation of the kidneys from surrounding tissue. Dynamic multi-slice FLASH images with a 17 s scan interval were recorded with TE 2 ms, TR 65 ms, FA 35°, 2 signal averages, matrix 128x128 and an acquisition voxel size of 0.31x0.31x1.0 mm3. In total, 100 dynamic images were recorded. Contrast agent (0.1 mmol/kg Gadovist) was injected via the tail vein at the start of the 1T dynamic phase.

Calibration. Using phantoms with Gd-concentrations ranging from 0 – 1.0 mM, DCE-MRI signal intensities were found to be linearly related to Gd-concentrations. All phantoms contained 0.1 mM MnCl2, to mimic a background T1 of muscle tissue (~800 ms). The linear coefficients as derived from the phantom experiment were used to convert in vivo signal changes to Gd-concentrations.

Pharmacokinetic model. Using the separable compartment model (SCM)3 the dynamic tissue concentration C(t) can be described as: C(t) = Vp Cp(t) + Fp e−t/τp @ Cp(t) with Vp the tissue plasma volume, Cp(t) the tissue plasma Gd concentration, Fp the tubular flow from the vascular into the tubular system, and τp the transit time of the contrast agent into the tubuli. Cp(t) can be obtained from the arterial Gd concentration Cd(t) according to Cp(t) = Tp e−t/τp @ Cd(t) where Tp is the transit time of the contrast agent in plasma. The plasma flow Fp from the artery into the tissue is given by Fp = Vp/TP. Fp is related to kidney perfusion, whereas τp is related to the glomerular filtration rate5. Since reliable estimations of the arterial input function in mice are difficult to obtain due to partial volume effects in small vessels and rapid circulation compared to the limited temporal resolution of DCE-MRI, Cd(t) was extracted from the signal in the erector spine muscle using a reference region model with vascular term6. K1, vP and vT of the reference muscle were set to 0.15 min−1, 0.1 and 0.025, respectively. Subsequent fitting of the tissue time curves by numerical optimization of the SCM in Matlab provided the independent parameters Tp, vP, vT, and Fp on a voxel-by-voxel basis. Voxels with a fit error greater than 50% were omitted from further analysis. Statistical analysis was performed using a paired samples t-test in SPSS 16.0.

Results
Examples of dynamic tissue Gd curves are shown in Figure 1. The clamped kidney clearly shows a stronger enhancement than the control kidney, which is likely due to endothelial cell damage resulting in vessel hyperpermeability. In addition, peak enhancement is reached at a later time point for the injured kidney, indicating a delayed plasma flow. This could be caused by vasoconstriction as a result of tubuloglomerular feedback or by an increased intrarenal resistance due to interstitial edema and/or vascular congestion, as also observed in human kidney transplants5. Figure 2 shows T2-weighted anatomical images of mouse kidneys with color overlays of the perfusion and filtration related parameters Fp and Tp, respectively. The injured kidney showed a marked reduction in both Fp and Tp. As an example, individual changes in Fp are shown in Figure 3. A clearly decreased Fp is observed in the injured kidney in almost all mice. A summary of all pharmacokinetic parameters is presented in Table 1. Statistically significant differences between clamped and control kidneys were only found for Fp and Tp.

Conclusions
Using DCE-MRI, a reduced perfusion and filtration could be observed in the injured kidney compared with the control kidney 24 hours post ischemia-reperfusion injury. The separable compartment model combined with a reference tissue input function allowed accurate fitting of the DCE-MRI data obtained in mouse kidneys. This method therefore seems suitable to investigate ischemia-reperfusion injury in a longitudinal fashion and to non-invasively determine the potential beneficial effects of novel drugs that protect the NHBD kidneys against ischemia-reperfusion injury.

References

Table 1. Pharmacokinetic parameters (mean ± sem).

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<thead>
<tr>
<th>Parameter</th>
<th>Clamped</th>
<th>Control</th>
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<tbody>
<tr>
<td>Fp (mL/min/100 cm3)</td>
<td>451 ± 24 *</td>
<td>481 ± 26</td>
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<tr>
<td>Fp (mL/min/100 cm3)</td>
<td>0.91 ± 0.15</td>
<td>1.04 ± 0.16</td>
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<tr>
<td>Tp (min)</td>
<td>0.039 ± 0.001 *</td>
<td>0.037 ± 0.001</td>
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<tr>
<td>Tp (min)</td>
<td>210 ± 91</td>
<td>301 ± 60</td>
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<tr>
<td>Tp (mL/100 cm3)</td>
<td>17.6 ± 1.1</td>
<td>17.7 ± 1.0</td>
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* P<0.05 compared with control kidney.