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 kidneys[1]. To improve the clinical outcome of these kidneys, novel therapies are being developed that limit ischemia-reperfusion injury[2]. 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 model[3] in combination with a reference region input function[4], 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. T_2-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 128×128 and an acquisition voxel size of 0.31×0.31×1.0 mm^3. 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 11th 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 $MnCl_2$ to mimic a background T_1 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: $C(t) = V_p C_p(t) + F_T e^{-t/T_T} \otimes C_p(t)$ with V_p the tissue plasma volume, $C_p(t)$ the tissue plasma Gd concentration, F_T the tubular flow from the vascular into the tubular system, and T_T the transit time of the contrast agent into the tubuli. $C_P(t)$ can be obtained from the arterial Gd concentration $C_A(t)$ according to $C_P(t) = T_P^{-1} e^{-t/T_P} \otimes C_A(t)$ where T_P is the transit time of the contrast agent in plasma. The plasma flow F_p from the artery into the tissue is given by $F_p = V_p / T_p$. F_P is related to kidney perfusion, whereas F_T is related to the glomerular filtration rate[3]. 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, $C_A(t)$ was extracted from the signal in the erector spinae muscle using a reference region model with vascular term[4]. K^{trans}, v_e and v_p 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 T_P, V_P, T_T, and F_T 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 transplants[5]. Figure 2 shows T_2-weighted anatomical images of mouse kidneys with color overlays of the perfusion and filtration related parameters F_P and F_T, respectively. The injured kidney showed a marked reduction in both F_P and F_T compared with the control kidney. As an example, individual changes in F_P are shown in Figure 3. A clearly decreased F_P 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 F_P and T_P.

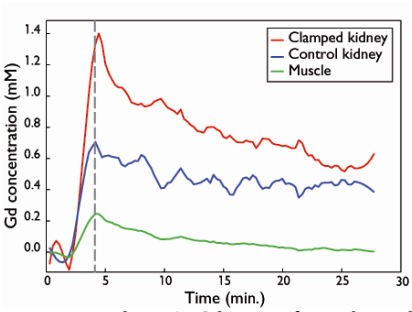

Figure 1. Mean dynamic Gd curves for a clamped, kidney, a control kidney and muscle tissue. The dashed grey line indicates peak enhancement of the control kidney.

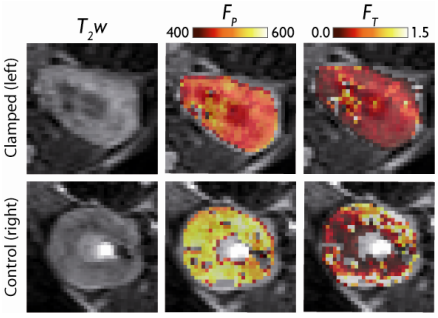

Figure 2. T_2-weighted images of mouse kidneys and corresponding color overlays of F_P (mL/min/100 cm^3) and F_T (mL/min/100 cm^3).

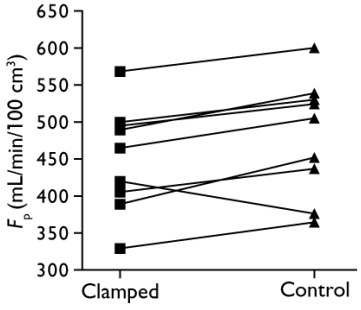

Figure 3. Individual F_P values for clamped and control kidneys.

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.

*Table 1. Pharmacokinetic parameters (mean ± sem). * P<0.05 compared with control kidney.*

	Clamped	*Control*
F_P (mL/min/100 cm^3)	451 ± 24 *	481 ± 26
F_T (mL/min/100 cm^3)	0.91 ± 0.15	1.04 ± 0.16
T_P (min)	0.039 ± 0.001 *	0.037 ± 0.001
T_T (min)	210 ± 91	301 ± 60
V_P (mL/100 cm^3)	17.6 ± 1.1	17.7 ± 1.0

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