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Pietro Irrera, Lorena Consolino, Juan Carlos Cutrin, Frank G. Zöllner and Dario Livio Longo

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Title

Dual assessment of kidney perfusion and pH by exploiting a dynamic CEST-MRI approach in an acute kidney ischemia-reperfusion injury murine model

Authors

Pietro Irrera, MSc^{1,2}, Lorena Consolino, PhD³, Juan Carlos Cutrin, PhD³, Frank G. Zöllner, PhD⁴ and Dario Livio Longo, PhD²

¹ Università degli Studi della Campania "Luigi Vanvitelli"

² Istituto di Biostrutture e Bioimmagini (IBB), Consiglio Nazionale delle Ricerche (CNR), Via Nizza 52, Torino, Italy

³ Centro di Imaging Molecolare, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università degli Studi di Torino, Via Nizza 52, Torino, Italy

⁴ Computer Assisted Clinical Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Corresponding Author

Dario Livio Longo, Istituto di Biostrutture e Bioimmagini (IBB), Consiglio Nazionale delle Ricerche (CNR), Via Nizza 52, Torino, Italia

Phone: +39-011-6706473, Fax: +39-011-6706487, email: dario.longo@unito.it, dariolivio.longo@cnr.it

Running Title

Dynamic MRI-CEST for simultaneous pH and perfusion in renal injury

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Abstract

Several factors can lead to acute kidney injury (AKI), but damage following ischemia and reperfusion injuries are the main risk factors and usually develop into chronic disease. MRI has often been proposed as a method with which to assess renal function. It does so by measuring the renal perfusion of an injected Gd-based contrast agent. The use of pH-responsive agents as part of the CEST (Chemical Exchange Saturation Transfer) -MRI technique has recently shown that pH homeostasis is also an important indicator of kidney functionality. However, there is still a need for methods that can provide more than one type of information following the injection of a single contrast agent and therefore furnish the improved and multiparametric characterization of renal function.

Herein, we propose, for the first time, dynamic CEST acquisition following iopamidol injection to quantify renal function by assessing both perfusion and pH homeostasis. The aim of this study is to assess renal functionality in a murine unilateral ischemia-reperfusion injury model at two time points (3 and 7 days) after AKI. The renal-perfusion estimates measured with iopamidol were compared with those obtained with a Gadolinium-based agent, via a Dynamic Contrast Enhanced (DCE)-MRI approach, to validate the proposed method.

Compared to the contralateral kidneys, the clamped ones showed a significant decrease in renal perfusion, as measured using the DCE-MRI approach, which is consistent with reduced filtration capability. Dynamic CEST-MRI findings provided similar results, indicating that the clamped kidneys displayed significantly reduced renal filtration that persisted up to 7 days after the damage. In addition, CEST-MRI pH imaging showed that the clamped kidneys displayed significantly increased pH values, reflecting the disturbance to pH homeostasis.

Our results demonstrate that a single CEST-MRI contrast agent can provide multiple types of information related to renal function and can discern healthy kidneys from pathological ones by combining perfusion measurements with renal pH mapping.

INTRODUCTION

Several different causes can lead to Acute Kidney Injury (AKI), with many being disease-dependent and generally involving decreased blood flow due to organ failure, injury and surgery^{1,2}. Others are consequences of direct damage to the kidneys, such as inflammation, allergy to drugs and nephropathy³. Ischemia/reperfusion injuries are the main events that lead to the establishment of AKI⁴, in addition to the obstruction of the urinary tract and acute tubular necrosis (ATN)⁵. Ischemia occurs when blood flow cannot freely enter an organ due to occlusion in the blood vessels, leading to an accumulation of waste products, hypoxic conditions and the start of inflammatory processes. The reperfusion event follows ischemia and occurs when the blood flow re-enters the organ, and the serum components recognize the inflammation. These two linked events occur frequently following kidney transplants, trauma injuries and embolism, and make AKI one of the leading causes of severe kidney problems, such as electrolyte dysregulation, drug toxicity and uremic complications⁵. For this reason, the development of robust, accurate and early diagnostic tools is mandatory. Currently, biochemical analyses are routinely used at the clinical level to evaluate kidney functionality (e.g. glomerular filtration rate) by assessing ureic nitrogen (BUN) and Serum creatinine (SCr) in the blood. They are quick and cheap analyses conducted on blood samples, but they can detect injuries only when consistent damage or consistent loss of function have occurred in the kidneys, and are not able to distinguish single kidney injuries⁶⁻⁹. This lack of ability to detect early damage, combined with the inability to distinguish which kidney is involved, is a severe limitation in current diagnoses. Other biomarkers that may be able to assess kidney injuries are currently under investigation and include cystatin C¹⁰, Neutrophil gelatinase-associated lipocalin (NGAL)^{11,12}, interleukine-18 (IL-18)¹³ and Kidney injury molecule (KIM-1), which are markers whose level increases following ischemic/nephrotoxic damage¹⁴. However, these promising biomarkers, which have yet to be approved in clinics, still do not provide information on single kidney activity, as they are quantified following urine collection.

Non-invasive imaging-based approaches are thus valuable tools for the evaluation of renal functionality. In particular, magnetic resonance imaging (MRI)-based approaches have been extensively investigated for their ability to assess several types of kidney information, including perfusion, oxygenation, cellular density and cell vitality, etc.^{15,16}. These methods all exploit changes in structural and physiological parameters that can be

indirectly detected by MRI, thus providing an accurate assessment of renal function, even at a clinical level¹⁷⁻
²⁵. Additional information can be obtained following the administration of contrast agents. In fact, measurements of the glomerular filtration rate (GFR) and renal perfusion can be obtained using the dynamic contrast enhanced (DCE)-MRI approach upon Gadolinium-based tracer injection in both preclinical and clinical investigations²⁶⁻³⁰. Furthermore, pH-responsive contrast agents have recently been proposed for use as part of the chemical exchange saturation transfer (CEST)-MRI technique to monitor pH values. This can be done by exploiting the pH dependence of the chemical exchange rate between water and mobile protons of the CEST molecule³¹⁻³⁶. Notably, one of these agents, iopamidol, has been used to measure renal pH values in several murine models of kidney injury, demonstrating that MRI pH imaging can act as a novel biomarker of kidney functionality, as it enables early damage and recovery to be detected, as well as being able to identify when only a single kidney is affected^{37,38}. These studies imaged *in vivo*, for the first time, the central role played by the kidneys in pH homeostasis and how marked renal pH changes can be detected following renal injury.

A common limitation of all the above-mentioned MRI-based techniques is that they can only provide a single type of functional information following the administration of an exogenous agent, and thus multiple contrast agent administrations are required for additional kidney interrogation. We have recently shown that iodinated contrast agents, besides providing pH readouts, are capable of perfusion estimates that are comparable to those obtained following the injection of small molecular weight Gadolinium-based complexes^{39,40}. In this study, we have investigated a CEST-based approach to the simultaneous assessment of both renal filtration and pH following the injection of a single contrast agent. This dynamic CEST-MRI approach after iopamidol injection has allowed us to longitudinally assess both kidney filtration and renal pH in a kidney ischemia reperfusion injury (KIRI) murine model. Iopamidol-derived perfusion estimates were compared with those obtained upon the administration of a conventional Gadolinium-based contrast agent. We have shown that our proposed approach provides **accurate and** multiple assessments of single kidney functionalities.

EXPERIMENTALS

Animal model

All animal experiments were approved by the University Ethical Committee and performed in accordance with European guidelines under directive 2010/63. Animals were purchased from Charles River Laboratories (Calco, Italy) and maintained in specific pathogen-free conditions. A unilateral renal ischemia-reperfusion injury model was performed on male BALB/c mice (n=7). After anesthesia with a mix of tiletamine/zolazepam 20 mg/kg/i.m. (Zoletil 100; Virbac, Milan, Italy) and 5 mg/kg xylazine i.m. (Rompun; Bayer, Milan, Italy), the left renal pedicle was reached by a medial laparotomy. Once exposed, a vascular clamp was applied for 30 min and then removed to produce a moderate-to-severe AKI in the left kidney ^{41,42}. After the clamp was removed, the kidney was inspected for the restoration of blood flow, which was proven when it returned to its original color. The wound was then closed with standard sutures. In order to maintain fluid balance, all mice were supplemented with 1 ml of saline, which was administered subcutaneously. All mice were examined by MRI before the ischemia reperfusion injury, as well as 3 days and 7 days afterwards.

***In-vivo* magnetic resonance imaging studies**

All MRI experiments were performed on a 7T MRI micro-imaging scanner (Avance 300, Bruker Biospin, Ettlingen, Germany). A 30-mm birdcage resonator was used for both transmission and receiving. In order to proceed with imaging, each mouse was anesthetized via the intramuscular injection of a mixture of tiletamine/zolazepam 20 mg/kg (Zoletil 100; Virbac, Milan, Italy) and 5 mg/kg xylazine (Rompun; Bayer, Milan, Italy). A catheter was inserted into the caudal vein to sequentially inject the two contrast agents, first iopamidol (Isovue, Bracco Imaging, Milano, Italy) at a dose of 2 g I/Kg body weight, and then, 15 min later, gadoteridol (ProHance, Bracco Imaging, Milano, Italy) at a dose of 0.1 mmol Gd/Kg body weight. The respiratory rate was continuously monitored using a respiratory air pillow (SA Instruments, Stony Brook, NY; USA). For the correct placement of the region of interest (ROI), kidneys were first localized in the three anatomical geometries with T₂-weighted images and then the anatomical reference image, a high spatial resolution T₂-weighted image (field of view of 3x3 cm², matrix of 256x256, slice thickness of 1.5mm, in-plane resolution of 117 μm), was acquired.

Dynamic CEST-MRI

Dynamic Contrast Enhanced CEST images were obtained by alternating 2 frequency offsets (± 5.5) ppm, with a saturation pulse of $6\mu\text{T} \times 1.5\text{s}$, and sampling time of 2s using a single-shot RARE sequence with centric encoding (typical setting TR/TE/NEX = 2.0 s/4.14 ms/1). Dynamic CEST images were acquired before (n=20) and after (n=130) the i.v. injection of iopamidol for a total of 10 minutes (150 images). To correct for B_0 inhomogeneities, a B_0 map was calculated from a full Z-spectrum acquisition (same for CEST-MRI pH imaging) before the dynamic CEST scan.

CEST-MRI pH imaging

After the dynamic CEST-MRI acquisition, a single-shot RARE sequence (TR/TE = 6s/4.1ms, Rare Factor = 96, centric encoding = 1) was used, preceded by a $3\mu\text{T}$ CW pre-saturation pulse for 5s and by a fat-suppression module, to acquire a full Z-spectra for pH imaging. A series of 43 frequencies were saturated to acquire a CEST spectrum in the frequency offset range ± 10 ppm. The acquisition time for a single CEST-MRI Z-spectrum was ca. 5 minutes. We used an acquisition matrix of 96×96 , reconstructed to 128×128 , with a field of view of $3 \times 3 \text{ cm}^2$ (in-plane spatial resolution = $234 \mu\text{m}$) and a slice thickness = 1.5 mm.

DCE-MRI

Subsequently, 15 min after CEST-MRI pH image acquisition, T_{1w} images were acquired using a gradient echo sequence with these settings: TE/TR/FA/NEX/Repetitions = 1.6ms/16ms/20°/2/150 for a total scan time of 10 minutes. The sampling protocol was kept the same as for the DCE-CEST protocol, with 20 images acquired before the ProHance injection and the remaining 130 after, for a total of 150 images (each T_{1w} image is acquired in 4s).

CEST analysis

All CEST images were analyzed using a homemade script implemented in MATLAB (The MathWorks, Inc., Natick, MA, USA). For dynamic CEST-MRI images, Saturation Transfer (ST) contrast was calculated by applying the asymmetry analysis on the images acquired at -5.5 and 5.5 ppm as:

$$ST = (SI(-5.5) - SI(5.5\text{ppm})) / (SI(-5.5\text{ppm})) \quad (1)$$

ST enhancements (STenh) curves were calculated as percentage changes between the ST before and after injection as $(ST_{\text{post-injection}} - ST_{\text{pre-injection}}) / (ST_{\text{pre-injection}}) * 100$ ⁵. Semiquantitative parameters (Peak, Slope and area under the curve - AUC) were obtained by applying a shape analysis to the CEST time curves^{43,44}. The peak represents the maximum ST enhancement over time, while the Slope is calculated by dividing the Peak estimated for the corresponding time post-injection of the peak. The AUC is calculated by integrating the ST enhancement time curve, from the injection time ($t = 40\text{s}$) to the end of the acquisition ($t = 600\text{s}$). For CEST-MRI pH mapping, the Z-spectra were interpolated, on a voxel-by-voxel basis, by smoothing splines, the B_0 -shift was corrected and the saturation transfer efficiency (ST %) was measured by punctual analysis⁴⁵. Kidney pH values were calculated by applying the ratiometric procedure at two frequency offsets, of 4.2 and 5.5 ppm, and back-calculated from the pH calibration curve, as described previously. **Errors in pH measurements were less than 0.1 pH units**³¹. The calculated parametric and pH maps have been superimposed onto the anatomical image.

DCE-MRI analysis

Signal intensity enhancements (SIenh) were calculated as percentage changes in signal intensities (SI) in T_{1w} -images as: $(SI_{\text{post-injection}} - SI_{\text{pre-injection}}) / SI_{\text{pre-injection}}$. The quantification of DCE-MRI kidney perfusion was performed using a pixel-by-pixel deconvolution approach⁴⁶, following previously described procedures⁴⁷⁻⁴⁹. Briefly, the following equation describes the deconvolution used to calculate the perfusion:

$$f = \max [C_t(t) \otimes^{-1} C_a(t)] \quad (2)$$

where $C_t(t)$ and $C_a(t)$ are the contrast agent concentrations, as a function of time, in a ROI in the tissue and inside the artery that feeds the region of interest (arterial input function, AIF), respectively. The perfusion, f , is the maximum value of the tissue impulse response function, i.e. the deconvolution (\otimes^{-1}) of the two concentration functions. Eq. 2 is solved by singular value decomposition (SVD) using a regularization of 0.15 times the maximal singular value⁵⁰.

Quantification was implemented using an in-house OsiriX plug-in^{51,52}. The software calculates maps of the renal blood flow (RBF), renal blood volume (RBV), and mean transit time (MTT). The calculated kidney perfusion estimates are systemically higher in healthy and diseased kidneys, but significantly different values are observed for normal kidneys than for diseased kidneys. The AIF was determined by carefully placing a ROI in the abdominal aorta to minimize partial volume effects due to the small vessel diameter. All data were normalized by subtracting the mean intensity of 20 baseline volumes, and the relationship between the contrast agent concentration and the measured signal intensities was assumed to be linear⁵³.

Histology

At the end of each experimental point, both kidneys were removed, cut along the sagittal axis and placed overnight in buffered 4% formaldehyde solution. Dewaxed 5 µm sections were haematoxylin-eosin stained. Ten randomized 200x fields from the cortical and the outer medulla zone were observed in order to evaluate the degree of tubular damage, in terms of cell necrosis, tubular dilatation and the presence of casts. The damage was scored as follows: 1 point for up to 30% of the tubules presenting alterations, 2 points for 31-60% and 3 points when more than 60% were damaged.

Statistical analysis

All values are expressed as mean \pm SD unless otherwise stated. Analysis of variance (ANOVA) and Bonferroni's multiple comparison test were used to compare the differences between the regions over the time points. A paired t test was performed to evaluate the differences between the contralateral and clamped kidneys for each time point. The Pearson product moment correlation test was used for correlation analysis between parameters. A value of $P < 0.05$ was considered to be statistically significant. Statistical analyses were performed using the Prism 5 program (GraphPad Inc, San Diego, California, USA).

RESULTS

Gd-enhanced dynamic curves

Dynamic Gd-enhanced curves showed a marked and similar sharp increase in signal intensity in the whole kidneys as well as in the cortical and medullar regions, while no differences were observed in the two kidneys of healthy mice (Figure 1A, left column). By contrast, three days after the ischemic damage, Gd-enhanced curves reported decreased enhancement in the clamped kidney, compared to the control (Figure 1A, middle column). Similar reduced enhancements were observed in both the cortex and outer medulla regions of the clamped kidneys following damage. Persistently reduced enhancement was observed in the AKI group up to one week after the damage; clamped kidneys showed smaller Gd-enhanced curves, reflecting decreased contrast-agent filtration (Figure 1A, right column).

DCE-MRI quantitative analysis

Figure 2 shows barplots of the estimated perfusion parameters MTT, RBF and RBV at all three investigated time points. Both perfusion (RBF) and blood volume (RBV) values are significantly reduced, compared to baseline values, three days after clamping. One week later, a moderate difference between the contralateral and ipsilateral kidneys was observed, although it was not statistically significant. The largest changes were recorded within the kidney cortex. At baseline, cortical kidney perfusion was $249 \pm 28 / 254 \pm 25$ ml/min/100ml, after clamping $217 \pm 60 / 140 \pm 78$ ml/min/100ml, and at follow up $205 \pm 35 / 150 \pm 42$ ml/min/100ml (contralateral/ipsilateral, respectively). Tables 1, 2 and 3 summarize the mean values of all perfusion parameters at several time points for the whole kidneys, the cortex and the medulla regions. Moreover, we noticed a slight decrease in RBF and RBV values in the contralateral kidneys following the damage. By contrast, no changes in the MTT estimate were observed over time and between the kidneys.

Figure 3 shows representative parametric maps of MTT, RBF and RBV superimposed on the corresponding morphological T_{2w} images at the different time points. Marked reduced perfusion and blood volume values over time are clearly visible for the clamped kidney. The mean transit time showed no visual decrease over time and between the contralateral and ipsilateral kidneys.

Dynamic CEST-MRI enhanced curves

The measured dynamic CEST-MRI enhanced curves showed a similar profile to those obtained following Gd injection, and hence reflect its capability to evaluate renal perfusion following the transit of iopamidol through the kidneys. In healthy mice, similar CEST-enhanced curves were observed in the right and left kidneys and the corresponding regions (Figure 1B, left column) in which the ROIs had been drawn. Dynamic CEST-MRI time curves, acquired three days after the ischemic damage, show remarkable differences in the clamped kidney, in comparison to the contralateral one. Significantly decreased values in the dynamic time curves can be observed for the clamped kidney, and for both the cortical and medullar regions, compared to the control kidney (Figure 1B, middle column). One week after the KIRI damage, the dynamic curves reveal that the filtration capability for the clamped kidney was drastically compromised, as their values are markedly reduced compared to the control ones (Figure 1B, right column). Overall, the dynamic CEST-MRI curves were able to distinguish the damaged kidneys from non-damaged ones by reporting differences in the measured time-curve profiles.

Dynamic CEST-MRI semi-quantitative analysis

Semiquantitative parameters were calculated from the dynamic CEST-MRI enhanced curves and showed equal values for both the left and right kidneys (and corresponding regions) in healthy mice. In particular, average values for the whole kidney regions were: 12.3 ± 2.3 and 12.3 ± 2.6 for the Peak, left and right kidneys, respectively; 800 ± 323 and 771 ± 355 for the AUC parameter, with a little variability being found among the members of the healthy group (Table 1, Figure 4). Moreover, parametric maps, superimposed onto the anatomical images, emphasized similar renal filtration in the left and right kidneys, with increased values when moving from the cortex to the calyx (Figure 5). Three days after ischemic reperfusion damage, semi-quantitative parameters for the clamped kidneys displayed a marked reduction in all the calculated estimates (Peak = 9.5 ± 3.4 / 7.7 ± 1.9 ; AUC = 972 ± 232 / 476 ± 157 , for contralateral and clamped kidneys, respectively; Figure 4). The parametric maps obtained from the shape analysis provided clear anatomical information about the extent of the damage in the clamped kidneys (Figure 5). One week after the damage, all of the parametric

estimates measured in the clamped kidneys were significantly reduced compared to the contralateral kidneys (Peak = 14.1 ± 2.2 / 10.4 ± 1.2 ; AUC = 939 ± 230 / 406 ± 121 contralateral vs. clamped, respectively; Figure 4C). Parametric maps showed the persistent damage in the clamped kidneys, which allows regions affected by reduced filtration to be easily distinguished (Figure 5C).

In order to compare perfusion estimates, regardless of absolute perfusion values, the ratios between diseased and healthy kidneys were calculated for both the DCE-MRI and dynamic CEST-MRI approaches (Figure 6). In comparison to baseline values, a marked and significant decrease in perfusion estimates was observed, based on the DCE-MRI approach (RBF and RBV), that was comparable with observations for the rAUC estimate, which was based on the dynamic CEST-MRI approach.

CEST-MRI pH imaging

In addition to the perfusion estimates, the CEST contrast agent can provide functional information about renal pH homeostasis. MRI-CEST pH mapping in healthy mice showed similar acidic pH values for both kidneys (average pH value = 6.7 ± 0.2 , Figure 7A). Representative renal pH maps are shown in Figure 7D; a homogenous pH gradient can be observed when moving from the cortex (more neutral) to the calyx (more acidic) for both kidneys, hence reflecting a similar acid-base balance. Compared to baseline values, the clamped kidneys displayed the alkalization of pH values, whereas the contralateral ones remained in more acidic conditions (pH = 7.1 ± 0.1 vs 6.7 ± 0.1 for clamped and contralateral kidneys, respectively $p < 0.001$; Figure 7A). Representative pH maps showed a reduction in the fraction of detected pixels three days after the AKI. This indicates reduced filtration and an increase in the pH values in all the regions of the clamped kidney. On the other hand, the entire contralateral kidney showed pixels with acidic values that are similar to the baseline (Figure 7E). One week after AKI, the clamped kidneys still showed slightly more alkaline values (6.7 ± 0.1 vs. 6.9 ± 0.2 for contralateral and clamped kidneys, respectively, $p < 0.05$; Figure 7A). Representative pH maps of a KIRI mouse demonstrate a reduced number of pixels in which the contrast agent is detectable, together with lower acidic values for the clamped kidney, in comparison to the contralateral one (Figure 7F).

A similar pH increase was observed for the mean values measured in the cortex and the medulla regions following ischemic damage (Figures 7B, C).

Figure 8 shows a linear regression analysis of the renal pH values and perfusion estimates obtained by either DCE-MRI (A) or from the dynamic CEST-MRI experiments (B). Strong and statistically significant correlation coefficients were obtained between renal pH and perfusion ($r = -0.93$, $P < 0.01$ for RBF and $r = -0.82$, $P < 0.05$ for rAUC, respectively).

Histologic evaluation

Kidneys showed different morphological patterns both three-days and seven-days post-ischemic injury. After three days of reperfusion, kidneys still exhibited signs of damage, i.e. cast deposition, tubular dilatation and interstitial inflammation. Casts, which were composed of amorphous hyaline material and cell debris, were observed from the cortical to the inner medulla, whereas dilated tubules and inflammation were more prominent in the inner cortex (Figure 9). The kidneys almost recovered their normal morphology seven days after the ischemic insult, but a few casts in the outer medulla and **scantly cortical tubules, with a slight degree of dilatation, were still present. (I'm not sure that "scantly" is the correct term here)** The observed damage score was 8 ± 1 three days after 30 minutes of ischemia, whereas the score was 3 ± 1 after 7 days.

DISCUSSION

In this study, we have developed a dynamic CEST-MRI approach to obtain *in-vivo* estimates of renal filtration from an analysis of dynamic CEST enhancement curves. Moreover, we have demonstrated, for the first time, that a single injection of iopamidol can provide two important types of information about renal function, such as filtration and pH, that can be calculated from dynamic CEST-MRI and CEST-MRI pH mapping, respectively. We can therefore detect and monitor renal function and pH balance in an all-in-one MRI analysis and this approach has been validated in a kidney ischemia reperfusion injury murine model. MRI-experiments were conducted in mice at two time points, after the KIRI damage, by applying a dynamic CEST-MRI

approach that was compared, in the same mouse and MRI session, with a DCE-MRI approach, which was based on the injection of a clinical Gd-based contrast agent. Semiquantitative parameters for the whole kidneys and anatomical regions (cortex and medulla), in the three groups of mice, were calculated from the shape analysis of CEST-MRI dynamic time-curves. For each parameter, we calculated the corresponding parametric map, on a pixel basis, showing the spatial distribution of the damage. The dynamic CEST-MRI approach has been demonstrated to be a suitable method to discriminate clamped kidneys from the contralateral ones, according to the reduced enhanced time curves observed after unilateral ischemia-reperfusion AKI. The severe impairment in the KIRI groups was proven by a corresponding decrease in all the semi-quantitative estimates, compared to the contralateral kidney and baseline values, that persisted up to one week after injury. Parametric maps provided anatomical information on the damage, in the form of a reduction in the detectable pixels in the injured regions of the kidneys, thus highlighting reduced renal functionality. The proposed dynamic CEST-MRI approach was validated by comparing CEST-enhanced curves with those obtained using the DCE-MRI approach following gadolinium injection. Dynamic CEST-MRI provided results that were similar to those obtained using the DCE-MRI approach, with time-curves reporting a decrease in signal intensity enhancements for the clamped kidneys, compared to the contralateral healthy kidneys and baseline profiles. The deconvolution analysis of the DCE-MRI time-curves showed a marked reduction in the calculated estimates, with the Plasma Flow parameter being the most sensitive to the reduced renal perfusion following AKI. Larger differences were observed for the estimates obtained from the dynamic CEST-MRI approach, compared to those from the DCE-MRI approach. This may be due to several factors, including contrast agents, analysis methods and origin of the contrast, although relative changes in the different metrics were comparable and showed similar trends (Figure 6).

Several studies, at the preclinical and clinical levels, have used the DCE-MRI approach to monitor renal perfusion and filtration with the extrapolation of clinically relevant parameters, such as renal blood flow and volume, mean transit time and glomerular filtration rate⁵⁴⁻⁶¹. In a similar animal model of kidney injury, DCE-MRI derived estimates, such as RBF and GFR, decreased upon ischemia/reperfusion injury after either deconvolution or two compartmental filtration model analyses^{26,48}. Furthermore, DCE-MRI has been demonstrated to distinguish pathological conditions earlier and with improved accuracy than standard biochemical markers in other models of kidney injury⁶²⁻⁶⁴. In a model of

renal artery stenosis, the calculated GFR and renal perfusion values were markedly reduced in stenotic kidneys, compared to control ones, and these results were in good correlation with FITC-inulin clearance results ⁶⁴. Another study, in human patients that underwent partial nephrectomy, estimated GFR using a compartmental approach for a single kidney and showed a decrease in single-kidney GFR after the operation. It was possible to identify the compensatory effect in the contralateral kidney ⁶⁵. A general decrease in these estimates can therefore be considered a clear indication of the reduced renal filtration that we demonstrated in this study and that can also be interrogated and monitored using a dynamic CEST-MRI approach. A major advantage of our study design was that all subjects underwent both dynamic CEST-MRI and DCE-MRI examinations in the same MRI session, allowing accurate correlations between the estimates derived from each method to be carried out.

In addition, CEST-MRI pH imaging provided meaningful information on renal pH homeostasis, with a clear distinction between injured and control kidneys in KIRI groups. These findings strongly support the view that the functional units appointed to guarantee pH homeostasis were compromised by the ischemia/reperfusion injury. The loss of these units seems to be irreversible (following an ischemic damage of 30 min), since the clamped kidneys are unable to recover normal pH homeostasis. Histopathological observations confirm the AKI condition, as a great number of the functional units in the clamped kidney were compromised and showed cast depositions, tubular dilatation and necrosis. Similar pH alterations (decreased renal acidification) have recently been reported to occur upon kidney damage in previous preclinical studies in different murine models. In particular, both kidneys were equally affected in a glycerol-induced bilateral injury model, and showed a marked increase in renal pH (up to 0.5 pH units) ³⁷. Furthermore, in a unilateral injury model that was induced by ischemia/reperfusion damage, alkalization occurred for the clamped kidneys with a pH increase that was markedly dependent on ischemia duration, whereas the contralateral ones reported acidic pH that was close to the physiological baseline condition ³⁸. Our results also demonstrate that both renal perfusion and pH homeostasis are affected by ischemia/reperfusion injury, and that there is a strong correlation between reduction in perfusion and the hampering of urine acidification.

In addition to iodinated-based CEST imaging, non-contrast or endogenous CEST-MRI based methods have also been exploited to assess kidney injuries. In a sepsi-induced kidney injury model, amide proton transfer

(APT) contrast showed a marked decrease in the injured kidneys, while, on the other hand, the same estimate did not report any change, compared to the contralateral kidneys, in a unilateral ureter obstruction model^{66,67}. Despite the advantage of not requiring an injection of exogenous contrast agents, the measured metrics are affected by variations in both concentration and pH that limit their specificity and accuracy⁶⁸. On the other hand, the administration of iodinated contrast media has to yet to be evaluated in patients with reduced kidney functionality, although several studies have shown reasonable safety even in patients with preexisting renal dysfunctionalities⁶⁹.

Our study has some potential limitations that merit further discussion. First, we only acquired a single slice that passed throughout the center of the kidneys, thus limiting the assessment of renal functionality to a small section of the renal parenchyma. Multi-slices and 3D CEST-MRI acquisitions have only recently been developed at a clinical level to cover the entire kidney volume, and the same can be said of accelerated acquisition strategies that are based on parallel imaging or compressed sensing, but these are still to be implemented in preclinical scanners⁷⁰⁻⁷². On the other hand, DCE-MRI studies have also been performed on a single central slice at the clinical level to improve temporal resolution and were able to give comparable values to whole-organ acquisition⁷³. Secondly, we have used a high field (7T) MRI scanner, which is not commonly found in medical centers, in order to better separate the two absorption frequencies of iopamidol. (please check meaning) However, it has previously been shown that accurate CEST pH imaging can also be obtained at lower fields (3-4.7T) or by using different ratiometric methods that exploit only one resonance, and that therefore have less stringent requirements for sufficient chemical shift separation⁷⁴⁻⁷⁶. Notably, CEST-MRI pH imaging has already been demonstrated to be feasible with clinical scanners operating at 3T^{77,78}. Thirdly, the implementation of more quantitative approaches for dynamic CEST acquisition are needed if perfusion values are to be compared with those based on DCE-MRI following the administration of gadolinium-based agents.

CONCLUSION

The findings presented herein strongly support the potential of the CEST-MRI approach to perform the combined assessment of renal filtration and pH homeostasis, and distinguish between damaged and contralateral kidneys, even at the single-kidney level. Thus, simultaneous *in-vivo* kidney pH and filtration imaging can be considered a potential imaging biomarker for the early assessment and monitoring of renal damage and can boast of promising translatability to clinical diagnosis.

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ABBREVIATIONS

AKI acute kidney injury

KIRI kidney ischemia-reperfusion injury

sCr serum creatinine

BUN blood urea nitrogen

CEST chemical exchange saturation transfer

ROI Region of interest

H&E haematoxylin and eosin

CA contrast agent

DCE dynamic contrast enhanced

Tables

Table 1. Semi-quantitative and perfusion analysis values for Dynamic CEST-MRI and DCE-MRI acquisitions in whole kidney

Kidney						
	Right			Left		
	Baseline	Post 3 days	Post 1 week	Baseline	Post 3 days	Post 1 week
DCE-CEST						
Peak	12 ± 2	10 ± 4	14 ± 2	12 ± 3	8 ± 2 ^{°°}	10 ± 1 ^{***}
rAUC	786 ± 286	972 ± 232	939 ± 230	746 ± 354	515 ± 177 ^{**}	406 ± 121 ^{***}
DCE-MRI						
MTT	18 ± 3	18 ± 6	19 ± 3	18 ± 3	17 ± 7	18 ± 2
RBF	199 ± 17	180 ± 53	177 ± 26	207 ± 24	107 ± 57 ^{°°}	147 ± 31
RBV	53 ± 8	46 ± 15	53 ± 9	56 ± 9	32 ± 15 ^{°°}	41 ± 8
^{°°°} indicates $p < 0.001$. ^{°°} indicates $p < 0.01$. (Bonferroni's test: baseline vs clamped)						
^{***} indicates $p < 0.001$. ^{**} indicates $p < 0.01$. (t-test: contralateral vs clamped)						

Table 2. Semi-quantitative and perfusion analysis values for Dynamic CEST-MRI and DCE-MRI acquisitions in cortex region

Cortex						
	Right (contralateral)			Left (clamped)		
	Baseline	Post 3 days	Post 1 week	Baseline	Post 3 days	Post 1 week
DCE-CEST						
Peak	11 ± 2	9 ± 3	12 ± 2	11 ± 2	7 ± 2 ^{°°°}	9 ± 1 [*]
rAUC	512 ± 208	758 ± 255	676 ± 258	533 ± 278	489 ± 142	352 ± 131 ^{**}
DCE-MRI						
MTT	14 ± 3	14 ± 4	17 ± 3	14 ± 2	13 ± 4	17 ± 1
RBF	249 ± 28	217 ± 60	205 ± 35	254 ± 25	140 ± 78 ^{°°}	150 ± 42 [°]
RBV	55 ± 9	48 ± 15	56 ± 6	57 ± 9	33 ± 16 ^{°°}	40 ± 9 [*]
^{°°°} indicates $p < 0.001$. ^{°°} indicates $p < 0.01$. [°] indicates $p < 0.05$. (Bonferroni's test: baseline vs clamped)						
^{**} indicates $p < 0.01$. [*] indicates $p < 0.05$. (t-test: contralateral vs clamped)						

Table 3. Semi-quantitative and perfusion analysis values for Dynamic CEST-MRI and DCE-MRI acquisitions in medulla region

Medulla						
	Right (contralateral)			Left (clamped)		
	Baseline	Post 3 days	Post 1 week	Baseline	Post 3 days	Post 1 week
DCE-CEST						
Peak	11 ± 2	9 ± 4	13 ± 2	12 ± 3	7 ± 2 ^{°°}	9 ± 0.5 ^{**}
rAUC	679 ± 299	990 ± 293	861 ± 234	712 ± 394	501 ± 198 ^{**}	422 ± 128 ^{***}
DCE-MRI						
MTT	20 ± 5	21 ± 9	20 ± 6	21 ± 5	19 ± 9	20 ± 3
RBF	168 ± 15	142 ± 45	151 ± 31	168 ± 27	86 ± 35 ^{°°°}	140 ± 32
RBV	50 ± 20	42 ± 16	42 ± 17	53 ± 22	35 ± 17	42 ± 23
^{°°°} indicates $p < 0.001$. ^{°°} indicates $p < 0.01$. (Bonferroni's test: baseline vs clamped)						
^{***} indicates $p < 0.001$. ^{**} indicates $p < 0.01$. (t-test: contralateral vs clamped)						

Figure Legends

Figure 1. DCE-MRI enhancement (SIenh%) times curves following gadoteridol injection (A) and dynamic CEST-MRI (STenh%) time curves upon iopamidol injection (B) for the whole kidneys (black) and for the cortex (red) and medulla (green) regions. No differences in time curve enhancements were observed for the kidneys of the healthy group (first column). After unilateral ischemia/reperfusion injury in the post 3 days (second column) and post 1-week (third column) groups, a marked decrease in enhancement time curves (for both DCE-MRI and dynamic CEST-MRI) was observed for all the regions of the clamped kidneys in comparison to the contralateral ones.

Figure 2. Column-bar graphs showing the results from the deconvolution analysis of DCE-MRI acquisitions following gadoteridol injection at different time points following AKI damage (Kidney: first column; Cortex: second column; Medulla: third column). Average values for the MTT (A), Renal Blood Flow (B) and Renal Blood Volume (C) estimates are reported for the whole kidneys, cortex and medulla regions with colour-filled column for contralateral kidneys (left) and pattern-filled column for ischemic ones (right). (* p value < 0.05; t-test contralateral vs clamped. ° p value < 0.05; °° p value < 0.01; °°° p value < 0.001; Bonferroni's test baseline vs clamped).

Figure 3. Representative images of the calculated parametric maps for MTT [s] (A), Renal Blood Flow [mL/min/100mL] (B) and Renal Blood Volume [mL/100mL] (C) obtained from the deconvolution analysis of DCE-MRI acquisitions upon gadoteridol injection at different time points following the AKI damage (baseline: first column; post 3 days: second column; post 1 week: third column). The arrow shows the clamped kidney.

Figure 4. Column-bar graphs showing the results from the shape analysis of Dynamic CEST-MRI acquisitions following iopamidol injection at different time points following AKI damage (Kidney: first column; Cortex: second column; Medulla: third column). Average values for the peak (A) and rAUC (B) estimates are reported with colour-filled column for contralateral kidneys (left) and pattern-filled column for ischemic ones (right). (* p value < 0.05; ** p value < 0.01; *** p value < 0.001; t-test contralateral vs clamped. ° p value < 0.05; °° p value < 0.01; °°° p value < 0.001; Bonferroni's test baseline vs clamped).

Figure 5. Representative images of the anatomical image (A) and of the calculated parametric maps for peak (B) and rAUC (C) obtained from the shape analysis of Dynamic CEST-MRI acquisitions following iopamidol injection at different time points following AKI damage (baseline: first column; post 3 days: second column; post 1 week: third column). The arrow shows the clamped kidney.

Figure 6. Ratios between left and right kidneys as estimated by DCE-MRI for MTT (a), RBF (b), RBV (c) or by dynamic CEST-MRI for peak (d) and rAUC (e) estimates for baseline (no ischemic, left) or after ischemia-reperfusion injury at 3 days (middle) and 7 days (right). (# p value < 0.05; ## p value < 0,01; #### p value < 0.001; Dunnett's test contralateral vs clamped).

Figure 7. Column-bar graph of measured CEST-MRI pH values for the whole kidneys (A), the cortex (B) and the medulla regions (C). Estimates are reported with colour-filled column for contralateral kidneys (left) and pattern-filled column for ischemic ones (right). Acidic values are observed at baseline for both the kidneys, whereas after ischemia-reperfusion injury the clamped kidneys showed higher pH values than contralateral ones. Representative pH maps (D-E-F for baseline, post 3 days and post 1-week groups, respectively) superimposed to the T_{2w} images showing neutral pH values for the clamped kidneys. The arrow shows the clamped kidney. (* p value < 0.05; *** p value < 0.001; t-test contralateral vs clamped. °°° p value < 0.001; Bonferroni's test baseline vs clamped).

Figure 8. Linear regression plots between CEST-MRI pH values and perfusion estimates obtained from DCE-MRI RBF estimate (in mL/min/100 mL, A) and dynamic CEST-MRI rAUC estimate (a.u., B) averaged for left and right kidneys and for the different time points (pre, post 3days, post 1week) after KIRI injury. Dashed lines denote regression lines.

Figure 9. Histological H&E stained sections for baseline (no ischemic) or after ischemia-reperfusion injury at 3 days and 7 days across the three renal regions (cortex, outer medulla and inner medulla) showing tubular injuries (‡= casts deposition, †= tubular dilatation, Δ= necrosis) for clamped kidneys compared to no ischemic one. (●= glomeruli).