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Volumetric contrast-enhanced ultrasound imaging of renal perfusion

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Abstract

Objective—Volumetric contrast-enhanced ultrasound (VCEUS) imaging has the potential to monitor changes in renal perfusion following vascular injury.

Methods—VCEUS utilizes a series of planar image acquisitions, capturing the non-linear second harmonic signal from microbubble (MB) contrast agents flowing in the vasculature. Tissue perfusion parameters (peak intensity, I_{PK} ; time-to-peak intensity, T_{PK} ; wash-in rate, WIR ; area under curve, AUC) were derived from time-intensity curve data collected during *in vitro* flow phantom studies and *in vivo* animal studies of healthy and injured kidney. For the flow phantom studies, either the concentration of MB contrast agent was held constant (10 $\mu\text{L/L}$) with varying volumetric flow rates (10, 20, and 30 mL/min) or the flow rate was held constant (30 mL/min) and the contrast agent concentration was varied (5, 10, and 20 $\mu\text{L/L}$). Animal studies were performed using either healthy rats or those that underwent renal ischemia-reperfusion injury. A series of renal studies were performed using healthy rats ($N = 4$) while the angle of the transducer was varied for each VCEUS image acquisition (reference or 0° , 45° , and 90°) to assess if repeated renal perfusion measures were isotropic and independent of transducer position. Blood serum biomarkers and immunohistology were used to confirm acute kidney injury.

Results—Flow phantom results revealed a linear relationship between MB concentrations injected into the flow system and the I_{PK} , WIR , and AUC perfusion measures ($R^2 > 0.56$, $P < 0.005$). Further, there was a linear relationship between changes in volume flow rate and the T_{PK} , WIR , and AUC metrics ($R^2 > 0.77$, $P < 0.005$). No significant difference was found between the transducer angle during data acquisition and any of the derived renal perfusion measures ($P > 0.60$). After induction of renal ischemia-reperfusion injury in a rat animal model ($N = 4$), VCEUS imaging of the injured kidney revealed an initial reduction in renal perfusion when compared to control animals followed by a progressive recovery of vascular function.

Conclusions—Preliminary results are encouraging and VCEUS-based renal perfusion imaging may prove clinically feasible for detecting and monitoring acute kidney injury.

Keywords

acute kidney injury; contrast-enhanced ultrasound; microbubbles; renal perfusion; volumetric imaging

INTRODUCTION

Medical ultrasound (US) has become increasingly popular in recent decades with continued improvements in imaging technique and resolution. US contrast agents are non-toxic gas-filled microbubbles (MBs) which exhibit non-linear oscillations upon exposure to an acoustic field. The harmonic backscatter can then be isolated from the backscattered signal through dedicated signal processing methods. Small enough to flow into microvasculature, MBs are able to enhance intensity of vasculature by up to 25 dB (1). Most recently, developments in MB usage have allowed for increased resolution (2), targeted imaging strategies (3–6), and potential therapeutic applications (7–10). Contrast-enhanced US (CEUS) has shown promise in detecting changes and identifying abnormalities in vascular perfusion at both planar (11–13) and volumetric levels (14–18). Other perfusion imaging options such as computer tomography (CT) or magnetic resonance (MR) imaging can expose the patient to ionizing radiation or nephrotoxic contrast agents. These qualities make volumetric CEUS (VCEUS) imaging an advantageous technique in comparison.

Acute kidney injury (AKI) is a life-threatening disease which involves the loss of kidney function with an increase in ischemia. The diagnosis is largely controversial, lacking a singular definition describing the disease, with mortality rates of approximately 50% (19). It has been suggested that the therapeutic outcome during treatment of AKI is directly related to the progress and severity of the disease (20). Current methods for quantifying AKI are searching for biomarkers indicative of kidney injury such as positive fluctuations in serum creatinine (21, 22). However, serum creatinine levels lack the sensitivity and specificity necessary for early detection (23). Due to the nephrotoxic nature of both CT and MR contrast agents, other standard perfusion imaging modalities are not suitable for diagnosis and monitoring of AKI. Currently, there are no methods to provide adequate information to fully diagnose extent of injury, therefore a reliable method of accurately quantifying the extent of AKI could be greatly beneficial to diagnosis, treatment regimens, and overall patient prognosis. The ability to visualize and quantify changes in perfusion in both specific regions and on the macroscopic scale of the kidney could provide useful supplemental information about this issue. VCEUS could provide a more detailed idea of the actual percentage of ischemic tissue resulting from this renal complication by identifying regions of ischemic tissue.

The focus of this paper is to investigate the repeatability and robustness of VCEUS imaging for tracking perfusion changes in the healthy and injured kidney. First, US imaging is examined in a vascular flow phantom to analyze macroscopic perfusion properties (changes) using variable blood flow rates and MB concentrations in a controlled environment. Next, experiments were performed imaging healthy rat kidneys as an investigation of homogeneous tissue to determine effects of frame reduction and the potential of VCEUS-

derived tissue perfusion parametric measurements to be consistent between different acquisition angles. Finally, preliminary work investigating vascular perfusion in a rat model of renal ischemia-reperfusion injury is presented with conclusions on the subject.

MATERIALS AND METHODS

Ultrasound imaging

VCEUS was performed using a SONIX Tablet US system (Ultrasonix Medical Corp, Richmond, BC, Canada). Real-time US imaging was performed utilizing a broadband 4DL14-5/38 volumetric transducer. A MB sensitive harmonic imaging mode (transmitting at 5 MHz and receiving at 10 MHz) was used at a low intensity (mechanical index < 0.1) to avoid MB destruction. Imaging settings, namely, volume scan rate, frame density, and field-of-view (FOV), were all held constant at 1.2 volumes/sec, 53 frames/volume, and 9.2° respectively for *in vitro* experiments, and 0.5 volumes/sec, 125 frames/volume, and 12.6° respectively for *in vivo* experiments to observe the entire kidney. Scan-converted data was acquired using Propello (Ultrasonix Medical Corp), a research-based software package. Scan converted data was analyzed using custom software developed in MATLAB (Mathworks, Natick, MA, USA) to process volumetric data sets. This was done through manual segmentation of a 3D region-of-interest (ROI), suppressing background signal using a threshold of 15 (out of a maximum intensity of 225), and obtaining the average voxel intensity within the ROI at each acquisition point to derive time-intensity curves for perfusion parameter estimation. Specifically, peak intensity (I_{PK}), time-to-peak intensity (T_{PK}), wash-in rate (WIR), and area under the curve (AUC) parametric measurements were estimated from time-intensity curve data describing the temporal history of MB flow. All experiments were performed in triplicate and Definity MB contrast agents (Lantheus Medical Imaging, North Billerica, MA, USA) were used in all contrast experiments.

In vitro experiments

Imaging was performed using a vessel-mimicking flow phantom (Model 524, ATS Laboratories, Bridgeport, CT, USA). This was a rubber-based phantom with acoustic properties similar to soft tissue, allowing repeatable measurements. A 6-mm diameter vessel embedded 1.5 cm below the surface of this flow phantom was used for all phantom experiments for characterization of macroscopic changes in vascular perfusion. A NE-1010 syringe pump (New Era Pump Systems, Farmingdale, NY, USA) was used to control volume flow rates of 10, 20, and 30 mL/min. These flow rates were chosen to provide varied flow speeds to validate the ability of VCEUS to track vessel fill rates and all used a constant MB concentration of 10 $\mu\text{L/L}$. The system was initially primed with MBs at concentrations of 5, 10, and 20 $\mu\text{L/L}$ of deionized water and then connected to a 60 mL syringe loaded with the same contrast concentration. These concentrations were chosen to provide a distinct difference in contrast for the vessel and used a constant flow speed of 30 mL/min. While they are much smaller than concentrations used *in vivo*, the consistent laminar flow environment of the flow phantom results in less MB destruction than the pulsatile stress in living systems. Placed 5.0 cm upstream of the imaging transducer, an external unfocused single-element transducer (Olympus Panametrics, Waltham, MA, USA) transmitted a 3 sec high-intensity pulse sequence (2.25 MHz, MI of 1.2) to destroy all flowing MBs. This

allowed for the system to mimic the relatively fast systemic perfusion rates that would be observed during kidney uptake of circulating MBs, as quickly after the end of the pulse sequence the bolus of MBs would enter the imaging transducer's FOV. Imaging was performed for 5 sec prior to MB destruction and for 1 min following to capture the recirculation of contrast with a FOV of 9.2° , 53 frames/volume and an imaging rate of 1.2 volumes/sec. This experiment was performed in triplicate for all flow rates and MB concentrations, as well as at an acquisition angle rotated 45° clockwise from the original imaging orientation to demonstrate the isotropy of VCEUS with respect to transducer orientation.

In vivo experiments

Animal model of healthy kidney—All animal studies were approved by the Institute of Animal Care and Use Committee (IACUC) at the University of Alabama at Birmingham. Four eight-week-old nude male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were anesthetized with 2.5% isoflurane gas. The dorsal skin region above the left kidney was covered in US coupling gel (Aquasonic, Warren, MI, USA) prior to all imaging studies. A syringe was drawn to contain 100 μL of contrast and 300 μL of saline and was loaded into a precision syringe pump (New Era Pump Systems) and MBs were injected through a tail vein catheter at a rate of 150 $\mu\text{L}/\text{min}$. Scanning was initiated immediately prior to contrast administration (21.6° FOV, 125 frames/volume, and 0.5 volumes/sec imaging rate) for a total duration of 2 min to capture MB circulation in the renal vasculature. This was performed in triplicate as well as at angles of 45° and 90° clockwise of the original imaging orientation to test for changes in perfusion parameters with respect to acquisition angle. At least 20 min was allowed to elapse between each US imaging session. Post-processing methods were performed as previously described. Also, faster volumetric imaging rates would allow for more accurate perfusion measurements (*i.e.*, improved temporal resolution). To that end, it is important to minimize the frame density of each volume acquisition, but not to the extent that spatial resolution is compromised. Post-acquisition, frame reduction was performed on healthy rat kidney VCEUS data by performing iterations of removing every alternate frame from an initial maximum frame density.

Animal model of acute kidney injury—Preliminary data was also obtained in an animal model of renal ischemia-reperfusion injury. Six male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) underwent baseline US imaging. Under aseptic conditions a complete right nephrectomy was performed via a right loin incision through established surgical procedures (24). Thereafter, three rats had a micro-serrefine vascular clamp placed to restrict all flow to the renal pedicle of the remaining kidney for a length of 30 min. The remaining rats underwent a sham treatment in which the remaining kidney was removed from the body cavity but no vascular restriction was applied. In both cases the kidney was kept moist with sterile gauze soaked in saline. Afterwards, the kidney was placed back into the body cavity, and the wound was sutured closed. Following surgery the rats were placed on a warming pad for 2 hr to allow recovery from the surgical procedures. VCEUS imaging was performed on the remaining kidney using the previously described methods at 5, 24, and 48 hr after surgery. Blood plasma was also collected at these time

points for analysis of serum creatinine changes associated with acute renal injury. Following the 48-hr time point, animals were humanely euthanized and the remaining kidneys were harvested for histologic analysis involving a hematoxylin and eosin (H&E) stain to identify tissue features of acute kidney injury.

Statistical analysis

Data was summarized as mean $\pm$ SE when applicable. A Pearson's correlation coefficient was used to analyze differences in time-intensity curve data at the various acquisition angles. A linear regression model was used to analyze perfusion parameter changes with respect to contrast concentration of flow rate in the flow phantom. ANOVA statistical testing compared perfusion parameter changes with angle in healthy kidney. All data was analyzed with SAS (SAS Institute Inc, Cary, NC, USA) and a p -value less than 0.05 was considered statistically significant.

RESULTS

During the flow phantom studies, either the concentration of MB contrast agent was held constant (10 $\mu\text{L/L}$) with varying volumetric flow rates or the flow rate was held constant (30 mL/min) and the contrast agent concentration was varied. Figure 1a shows time-intensity curves for three different MB concentrations for a fixed volumetric flow rate. While all three time-intensity curves reach their peak intensity at the same time point, there is an obvious correlation of peak intensity to contrast agent concentration which is further described in Figure 2a. Also, the derived perfusion parameters from time-intensity curve data (*i.e.*, I_{PK} , T_{PK} , WIR , and AUC) are described in Figure 1a. WIR was calculated by the slope from the data points 10% to 90% of the maximum value of the wash-in curve, AUC was calculated as the integral of the time-intensity curve, and T_{PK} was time taken to reach I_{PK} . For the 5 $\mu\text{L/L}$ time-intensity curve the parametric perfusion values for I_{PK} , T_{PK} , WIR , and AUC were found to be 17.5 ± 1.1 , 4.4 ± 0.4 sec, $3.9 \pm 0.6 \text{ sec}^{-1}$, and 205.1 ± 14.9 respectively. Figure 2 describes how perfusion parameters are influenced by changes in MB contrast agent concentration and volumetric flow rate. I_{PK} revealed a linear relationship with the concentration of contrast agent used (Figure 2e, $R^2 = 0.56$, $P < 0.001$), as did WIR (Figure 2g, $R^2 = 0.75$, $P < 0.005$) and AUC (Figure 2h, $R^2 = 0.93$, $P < 0.001$). T_{PK} (Figure 2b, $R^2 = 0.93$, $P < 0.001$), WIR (Figure 2c, $R^2 = 0.92$, $P < 0.001$), and AUC (Figure 2d, $R^2 = 0.77$, $P < 0.005$) had linear relationships with changes in flow rate. As expected, there was also no significant change in T_{PK} with respect to contrast concentration (Figure 2f, $P > 0.85$) as well as I_{PK} with respect to flow speed (Figure 2a, $P > 0.06$). Similar results were obtained from the same experiments with the imaging transducer rotated 45° clockwise from the original (reference) acquisitions. Because of the limitations of the single vessel flow phantom acquiring VCEUS data at 90° was not possible. This is due to the fact that volumetric US probes generally overestimate flow speeds at this angle due to the sweeping nature of data collection in relation to the laminar flow inside the flow phantom.

Figure 3 illustrates the time intensity curves obtained at different transducer orientations: 0° (origin), 45°, and 90°. Importantly, a strong correlation was found between time intensity curves acquired at the various transducer scanning angles ($\rho > 0.98$, $P < 0.001$). The

accompanied images are a set of representative volumetric renderings illustrating the temporal kinetics of renal perfusion after systemic administration of MB contrast agent. Summary data suggests the isotropic nature of volumetric US acquisitions. This is further examined in Figure 4 which describes the perfusion parameters versus changes in image acquisition angle. There was no statistical difference between all three angles in any of the four parameters investigated ($P > 0.48$). This suggests that with proper ROI analysis it is possible to discern repeatable perfusion parameters *in vivo* regardless of transducer angle, demonstrating robustness when using VCEUS imaging in longitudinal studies.

Figure 5 compares representative perfusion measurements in healthy rat kidneys at different frame rates. The maximum frame density permitted using the VCEUS imaging system had a step size (frame separation) of 0.173° and each subsequent point was obtained by using half the frame rate of the previous point, down to a separation of 2.768° per frame. At frame separations of 1.384° or larger, there was a significant difference ($P < 0.05$) in I_{PK} perfusion measures compared to those obtained at the starting maximal frame density (*i.e.*, 0.173°). Similar patterns were observed for the T_{PK} , WIR , and AUC parametric perfusion measurements indicating that spatial resolution is an important consideration when performing VCEUS imaging and that representative planar images may not be sufficient to fully characterize tissue perfusion levels throughout the entire kidney.

Figure 6 depicts the relative difference between the percent change of the mean values for each of the perfusion measurement obtained in control kidneys and those subjected to acute ischemic conditions. Early US measurements indicate that perfusion was considerably lower in the injured kidneys. Specifically, the I_{PK} parametric measurement was the most disparate at the 5-hr time point and most similar at the 48-hr time point. This data suggests that post-surgery the injury group was less perfused than the control group, and by the 48-hr time point limited reperfusion had occurred. Similar results were found for T_{PK} , WIR , and AUC demonstrating a peak difference at either the 5-hr or 24-hr time point with an increase in similarity to control measures by the 48-hr time point. This was also supported by serum creatinine levels, as shown in Figure 7, where there was a peak in the difference between mean serum creatinine values from control rats and rats subjected to ischemic injury at the 24-hr time point and then had partially recovered by 48 hr. Histology was also performed on the control and injured rat kidneys at 48 hr post-surgery. As shown in Figure 8, there was evidence of tubular cast formation and tubular necrosis in the group that underwent ischemia/reperfusion injury, validating that vascular injury had occurred. This indicates that there was a reduction of blood flow, supporting our previous findings.

DISCUSSION

Enhancing methods for detecting severity of AKI has the potential of improving patient outcome by increasing the information, knowledge and understanding of how to treat the disease. The strategies investigated here are intended to supplement current methods to gain a more informative description of AKI where portions of the tissue become ischemic. For non-homogenized tissues it is important to obtain the same information at each time point in a longitudinal study because variation between time points can vary to a large degree just from transducer misalignment (14, 18). Therefore, transducer placement is integral between

time points for reliable data. VCEUS reduces error from misplacement by collecting entire volumes in real-time. While VCEUS has been successful in tracking vascular changes in a longitudinal study (15), we were able to show the robustness of this method in terms of acquisition angle for both flow phantom and rat kidney imaging. It is possible to obtain repeatable measurements, even in extreme cases of up to 90° misalignment as demonstrated in Figure 3. While in practice, one would strive for the transducer orientation to be the same at each acquisition point, the data presented in this study shows that small misalignments utilizing VCEUS should have very small effect on the data acquired, demonstrating improvements over planar US.

Imaging in a flow phantom allowed for a controlled environment to analyze the ability of VCEUS in tracking macroscopic changes in perfusion. *In vivo*, it is possible to ascertain visual changes in perfusion levels after producing 3-dimensional renders of the collected data as seen in Figure 3. This has possible applications as a non-invasive modality to help monitor kidney function following procedures associated with kidney failure such as cardiac surgery, liver transplant, or kidney transplant. Also, we were able to determine that a maximum distance between frames without significant loss of temporal resolution was 0.692°. Reimaging at this step size would increase the frame rate of the acquisition by approximately a factor of four, allowing for a more accurate interpretation of time-intensity curve data as more data points could be collected in the same time window.

The preliminary data obtained from the ischemia-reperfusion experiments was limited due to complications experienced during surgery. From the initial eight rats, one rat from each group died following the procedure due to a high stress burden on their system, and one of the rats in the injury group was found to have very limited reperfusion following surgery and having greater injury than expected. More extensive experiments analyzing a larger variety of degrees of AKI need to be completed to fully understand the efficacy of VCEUS in investigating the matter. Despite these facts, the results shown in Figure 6 were promising. This is consistent with data collected using 99mTc-MAG3 in gamma-camera imaging, whereby renal perfusion was shown to be slowly restored to the damaged kidney (24). The data observed in the T_{PK} , WIR , and AUC perfusion measurements also support the trend that kidney function is returning by the 48-hr time point in the kidney injury group. Data from the T_{PK} and WIR perfusion measurements showed a peak at the 24 hr time point with a return to normal function by the 48 hr time point, possibly suggesting that post-trauma inflammation increased the time necessary for the tissue to perfuse. This data is also supported by the serum creatinine levels as shown in Figure 7. We found the serum creatinine levels to peak in the injury group at 24 hr and then partially recover by 48 hr. This suggests that serum creatinine levels continued to build up after 5 hr to reach its highest levels around 24 hr. The drop in levels that occurs by 48 hr then suggests that the renal tissue had begun to undergo reperfusion.

CONCLUSIONS

Acute kidney injury is a serious disease in need of more methods to help diagnose extent of injury and monitor the tissue throughout disease progression. Data and preliminary work described shows that further examinations of these methods have the potential of increasing

the understanding of how the disease progresses on a patient to patient basis. This data suggests that transducer angulation for VCEUS does not affect time-intensity curves or the resulting perfusion parameters derived from them. Also, a step size of 0.692 degrees is an adequate tradeoff between acquisition speed and data accuracy during VCEUS data acquisition for the methods presented here. Successfully identifying ischemic regions through future studies could potentially improve treatment strategies and boost patient prognosis.

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Figure 1.

Representative results from the flow phantom VCEUS imaging study. (a) Time intensity curves of the first 12 seconds acquired at flow rates of 30 mL/min and using MB contrast agent concentrations of 5, 10, or 20 µL/L. Perfusion parameters derived from time-intensity curve analysis are illustrated for the 5 µL/L dataset. (b) Representative 3D renders of the flow phantom vessel describing the onset and temporal evolution of vascular flow.

Figure 2.

Changes in perfusion parameters derived from image data collected using either a constant MB contrast agent concentration of 10 $\mu\text{L/L}$ with changes in flow rate (a–d) or using a fixed flow rate of 30 mL/min and varying contrast agent concentration (e–h). Bar graphs describe the following perfusion parameters: (a, e) I_{PK} , (b, f) T_{PK} , (c, g) WIR , and (d, h) AUC .

Figure 3.

(a) Illustration of transducer orientation and the three different angulations used for VCEUS imaging of the same healthy rat kidney. (b) Time-intensity curve data acquired in healthy rats using VCEUS imaging and relative transducer angles of 0° (reference), 45°, and 90°. (c) Representative 3D renders of a healthy kidney displaying progressive perfusion throughout the whole organ following systemic administration of a MB contrast agent.

Figure 4. Changes in perfusion parameters with respect to angle of acquisition: (a) I_{PK} , (b) T_{PK} , (c) AUC , and (d) WIR .

Figure 5.

Percent change in (a) I_{PK} , (b) T_{PK} , (c) WIR , and (d) AUC perfusion measures from the maximum frame density allowed for our VCEUS system and as the frames per volume (*i.e.*, spatial resolution) are progressively reduced. Note imaging rate and temporal resolution were held constant.

Figure 6.

Results from VCEUS imaging of control kidneys (sham surgery) and kidneys that underwent ischemia/reperfusion injury in a rat animal model. The bar graphs detail temporal differences between injured and control kidney for (a) I_{PK} , (b) T_{PK} , (c) WIR , and (d) AUC perfusion measurements from baseline (pre-surgery). Data trends indicate that perfusion levels in injured kidneys are slowly being restored and approaching that observed in the control kidneys.

Figure 7.

Blood serum creatinine levels collected at time points concurrent with VCEUS imaging sessions. Bar graph details temporal differences between injured and control kidney biomarker measurements and indicates that the injured kidneys are slowly recovering from the short-term ischemic conditions.

Figure 8.
Representative histological results from the (a) control and (b) injured kidney groups.
Formation of tubular casts (indicated with C) and tubular necrosis (indicated with N) were
evident in the injury groups.