High-resolution renal perfusion mapping using contrast-enhanced ultrasonography in ischemia-reperfusion injury monitors changes in renal microperfusion

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Alterations in renal microperfusion play an important role in the development of acute kidney injury with long-term consequences. Here we used contrast-enhanced ultrasonography as a novel method for depicting intrarenal distribution of blood flow. After infusion of microbubble contrast agent, bubbles were collapsed in the kidney and postbubble destruction refilling was measured in various regions of the kidney. Local perfusion was monitored *in vivo* at 15, 30, 45, 60 minutes and 24 hours after 28 minutes of bilateral ischemia in 12 mice. High-resolution, pixel-by-pixel analysis was performed on each imaging clip using customized software, yielding parametric perfusion maps of the kidney, representing relative blood volume in each pixel. These perfusion maps revealed that outer medullary perfusion decreased disproportionately to the reduction in the cortical and inner medullary perfusion after ischemia. Outer medullary perfusion was significantly decreased by 69% at 60 minutes postischemia and remained significantly less (40%) than preischemic levels at 24 hours postischemia. Thus, contrast-enhanced ultrasonography with high-resolution parametric perfusion maps can monitor changes in renal microvascular perfusion in space and time in mice. This novel technique can be translated to clinical use in man.

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The pathophysiology of acute kidney injury (AKI) involves tubular injury, inflammatory processes, and changes in renal microvascular perfusion,[1] which result in a generalized or localized impairment of oxygen and nutrient delivery to, and waste product removal from, cells of the kidney.[2] The postischemia perfusion to the outer medulla is decreased disproportionately to the reduction in total kidney perfusion in animals and likely in patients following ischemic injury.[3–7] The local perfusion in the outer medulla can be reduced due to arteriolar vasoconstriction, endothelial injury, and local interstitial edema secondary to increased capillary permeability. This can result in tubular injury and interstitial inflammation, which, in turn, can lead to decreased capillary density, chronic tissue hypoxia, and eventually fibrosis.[6,8] Patients at risk for AKI often receive a combination of i.v. fluids and vasoconstrictive agents, which can further decrease local perfusion and increase interstitial edema.[6] Therapeutic approaches, guided by impaired intrarenal perfusion and localized intrarenal edema, are not possible due to the absence of suitable bedside diagnostic and treatment monitoring technologies for detecting alterations and distributional deficiencies in renal microvascular perfusion.

RESULTS AND DISCUSSION

We have applied contrast-enhanced ultrasonography (CEUS) to the assessment of the distribution of renal microperfusion in space and time in mice subjected to ischemia-reperfusion injury (IRI). Whereas CEUS has been used to provide information regarding cortical perfusion,[9–12] to our knowledge there is no CEUS study that reliably demonstrates perfusion changes in the outer medulla. One reason why outer medullary perfusion could not be adequately assessed is interference with signals from large vessels. Another limitation of previous studies[13] is the use of reperfusion time to reach a percentage of quasi-steady-state blood flow. In AKI, when many outer medullary capillaries may be without flow, this parameter will not reliably reflect regional blood flow because the quasi-steady-state flow may be very impaired and yet the time to reach a specific percentage of that low steady-state flow unchanged.

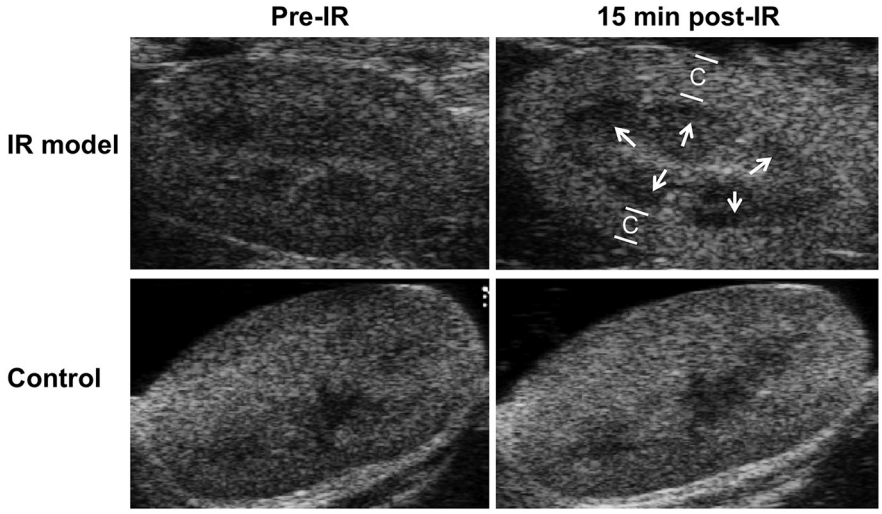

Figure 1 | B-mode ultrasonographic images showed dark regions under the cortex at 15 minutes post-IR. Male BALB/c mice were subject to 28 minutes of bilateral ischemia by clamping the renal pedicles. Animals were imaged with B-mode, brightness mode, ultrasonography at 15, 30, 45, and 60 minutes, and 24 hours after reperfusion. At 15 minutes after ischemic reperfusion (post-IR), a dark region was found under the cortex (C) in the examined animals. The dark band was not seen in any pre-IR or 24-hour post-IR image. Control animals not exposed to IR did not show the dark band at any time point.

In our study we used parasagittal transducer orientation, in which the large vessels are perpendicular to the imaging plane and do not disturb the visualization of the microvasculature in the outer medulla. In addition, for data analysis, we used plateau image intensity values. This reflects the actual microbubble delivery (microvascular blood flow) to the given location. During a 2-minute intravascular infusion, the microbubbles are collapsed using high mechanical indices (MI) ultrasound bursts and recovery is monitored over time in various regions of the kidney. High-resolution parametric perfusion maps were developed to detect and monitor microvascular perfusion changes in space and time, providing insight into the distributional changes in the microvascular perfusion, over space and time, in the kidney following IRI.

Mice were imaged at baseline and at various times after 28 minutes of bilateral ischemia with B-mode ultrasonography. A dark region secondary to decreased echogenicity was present under the kidney cortical area in images taken after 15 minutes of reperfusion (Figure 1). In the first hour after the clamp release, this dark band became progressively less visible, and at 60 minutes postischemia-reperfusion (post-IR), it was no longer apparent. The dark band was not seen in any pre-IR or 24-hour post-IR image or at any time point in control (sham) animals. There are 2 possible reasons for the brighter appearance of the post-IR cortical image: (i) The manipulation around the renal pedicles caused the elongation and modification of subcutaneous fat; and (ii) a small amount of contrast agent may remain from the first contrast infusion, although this is unlikely because the post-IR B-mode image was taken 43 minutes later (28 [ischemia duration] +15 [waiting time post-IR] = 43 minutes). The half-life of the contrast agent is short (<5 minutes).

To estimate whole kidney and regional microvascular perfusion, high-resolution parametric perfusion maps of the kidneys were collected to detect distributional change in the microvascular perfusion. These are presented in pseudocolor (Figure 2). Although there was some variability among animals, the perfusion maps clearly showed regional differences in microvascular perfusion of the post-IR kidney. The parametric maps revealed the most prominent perfusion loss in the outer medulla as early as 15 minutes after reperfusion (Figure 2). The dark regions under the cortical region (outer medulla) that were found on B-mode images (Figure 1), reflecting reduced local perfusion, were located at the same regions on the parametric maps. On the 24-hour post-IR images, delayed perfusion recovery persisted in the outer medulla. One animal, mouse #IR7 developed more severe injury in response to the 28-minute ischemia. The rapid and marked reduction in the microvascular perfusion can be identified on the parametric perfusion maps at post-IR 30, 45, and 60 minutes. This animal died post-IR 24 hours. No such spatial distributional change was found in any of the control animals (Figure 2).

Representative single kidney perfusion tracings for the whole kidney (Figures 3 and 4), or cortical, outer, and inner medullary regions (Figures 5 and 6) were obtained for ischemic and control animals, by monitoring refill-induced nonlinear signals of intact microbubbles after acutely collapsing them with high MI ultrasound bursts. After the removal of renal pedicle clamps, the microvascular perfusion gradually decreased in the whole kidney over the first hour post-IR (average perfusion decrease in the whole kidney was found as 50%, $P = 0.0006$, $n = 12$) as reflected by lower echo intensity plateau value at baseline at preburst and after burst. At 60-minute post-IR, perfusion was decreased in the cortex and inner medulla but mostly in the outer medulla (by 69% vs. baseline pre-IR; $P = 0.0001$). Whereas cortical and inner medullary perfusion returned to levels close to baseline by