The biological response of rodent kidneys to low frequency, full volume diagnostic contrast-enhanced ultrasound imaging: Pilot data

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Abstract

With the growth of contrast-enhanced ultrasound (CEUS) clinically, there are concerns about histologic bioeffects in regards to the implementation of high mechanical index (MI) imaging, such as the imaging sequence used for a specific CEUS technique known as flash-replenishment. The data presented are results from a pilot study, which explored flash-replenishment with high and moderate MI imaging sequences at time points of 24 hours and 2 weeks post imaging. This pilot study was followed by a larger study, which can be found in a journal article entitled “Histological and Blood Chemistry Examination of the Rodent Kidney After Exposure to Flash-Replenishment Ultrasound Contrast Imaging” Nyankima et al., 2019.

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1. Data

Data demonstrates the histopathologic and blood urea nitrogen (BUN) changes after the use of flash-replenishment contrast-enhanced ultrasound (CEUS) in the rodent kidney. Fig. 1 frames the bioeffect indicators and time table of the data presented. Fig. 2 presents the results of histopathology assessment. Histopathology, the standard for assessing kidney bioeffects, was quantified as red blood cells (RBCs) in the glomeruli or tubules, which denote signs of hemorrhage in kidney tissue at the final time point. Lastly, BUN levels were measured as a global indication of kidney health, and were measured in serum isolated prior to imaging and at the time of necropsy. Fig. 3 depicts these results in graphical format. Table 1 and Table 2 present the raw values of BUN levels through several time points before and after flash-replenishment imaging. Table 1 includes the raw values of data presented in Fig. 3. Table 3 and Table 4 report raw values of the histopathology assessment at different time points before and after CEUS. Table 3 presents values presented in Fig. 2. Tables 2 and 4 present data published in Nyankima et al. [1].

2. Experimental design, materials, and methods

A pilot study was conducted with an N of 15 (n = 3–4/group) as a first round assessment of microbubble-induced bioeffects during CEUS imaging. This study was conducted with the same instrumentation and animal subjects as the final study presented in Nyankima et al. (2019) [1], but with a few differences in parameters. Female Fischer 344 rat kidneys were chosen as the in vivo model for the study. All procedures were approved by the University of North Carolina at Chapel Hill Animal Care and Use Committee board prior to beginning the study. Animals were separated into groups as demonstrated in Fig. 1.
Imaging was conducted using an Acuson Sequoia 512 (Mountain View, CA, USA), with a 4C1 curved array transducer. Definity microbubbles were purchased from Lantheus (North Billerica, MA, USA), and used for the study. Animals were anesthetized with isoflurane, and placed on their side while imaging in the sagittal plane. Microbubbles were administered in a tail vein at a dose of 200 μL of Definity in 400 μL of 0.9% saline. The solution was continuously infused into the animal at a rate of 40 μL/min.

Flash-replenishment imaging was conducted across the kidney volume in 1mm step sizes across a 1.5cm volume. In each plane, a microbubble destructive pulse was conducted at 3 MHz, a frame rate of 10 Hz, and an MI of either 1.9 or 1.0. This flash pulse was followed by low MI imaging pulses at 1.5 MHz, 14 Hz frame rate, and an MI of 0.2. Low MI imaging was completed for 1 second, before moving to the subsequent plane, to allow for microbubble perfusion after destructive pulses. Animals were euthanized at either a time point of 24 hours or 2 weeks. At this time, kidneys were prepared for histopathology assessment.

In this setup, the control kidney was exposed to ultrasound and bioeffects were observed in both. To address these issues, changes were made for the full study, including re-positioning the rat to reduce control kidney exposure.

2.1. Preliminary observation: histopathology at 24-h and 2 weeks

Histopathologic assessments were conducted on hematoxylin and eosin (H&E) stained tissue. Quantitative analysis was conducted by counting RBCs in glomeruli or tubules, a sign of hemorrhage, in both the experimental and control kidneys. No RBCs were identified in the glomeruli. The total number of RBCs in tubules, or RBC casts, for both control and experimental kidneys are presented in Fig. 2. In rats exposed to 1.0 MI pulses for CEUS imaging, no evidence of hemorrhage was found at either the 24-h or 2-week time points (see Fig. 2). In the 1.9 MI group, total RBC cast score averaged 13 ± 9 (mean ± SEM) when assessed after 24 hours. These signs of hemorrhage were absent on histopathologic examination after 2 weeks (see Fig. 2). As a result of the imaging setup, the signs of hemorrhage were observed in both the right and left kidney, removing a control from this study. It was this finding in the 1.9 MI at 24 hours that led to the final (and larger) round of data collection. To eliminate the chance of ultrasound exposure in the control kidney, the animals were imaged on their back, and the transducer mechanically-steered over the chosen imaging side. To determine if RBCs were originating from the glomeruli as a result of glomerular capillary hemorrhage, an earlier time point of 4 hours was chosen since urine progresses from the glomeruli to the tubules.

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![Fig. 1. Experiment Timeline.](image-url)

Prior to imaging, BUN levels were measured (t = 0). Short term bioeffect assessments were quantified 24 hours after imaging. Blood and kidneys were collected for BUN and histopathology analysis. Long term bioeffect assessments were quantified 2 weeks post imaging. This timeline was followed for animals exposed to 1.0 MI and 1.9 MI microbubble destructive pulses. This figure was made in likeness of Fig. 1 of Nyankima et al. [1] and captures the difference in time points assessed.
2.2. Preliminary observation: kidney clinical chemistry at 24-h and 2-weeks

Blood samples prior to imaging (baseline) and at the time of necropsy (endpoint) were measured for BUN. For subjects exposed to an MI of 1.0, there was no significant change in the BUN levels from baseline to their endpoint (Fig. 3).

In the 1.9 MI group, no significant change was observed after 24 hours. At the 2-week endpoint, the BUN of the 1.9 MI group had significantly increased from $17 \pm 1$ mg/dL at baseline to $23 \pm 2$ mg/dL at 2 weeks ($P = 0.002$).

The normal BUN range for female Fischer rats is $19.18 \pm 2.39$ mg/dL (95% confidence interval $~15–24$ mg/dL) [2]. As indicated in Fig. 3, mean BUN levels of all four groups remained in this range.

![Clinical Chemistry Results](image)

**Fig. 3. Clinical Chemistry Results.** BUN levels are grouped by imaging parameters and endpoint. Results of paired T-test indicate a statistically significant increase in BUN levels for subjects in the high MI group after 2 weeks ($p$-value from 0.001 to 0.01 is marked with a ‘**’). No statistical significance was observed in the low MI groups. Average BUN values remained within normal range for female Fischer rat (15–24 mg/dL, dashed lines).
2.3. Raw data: kidney histopathology and clinical chemistry

Table 1
Clinical Chemistry Results. Raw data of blood urea nitrogen measurements taken from groups exposed to an MI of 1.9 or 1.0 (N = 15). Blood was collected prior to contrast imaging (baseline) and at the final time point of either 24 hours or 2 weeks.

| Subject | High MI | Moderate MI |
|---------|---------|-------------|
|         | Baseline | 1.9 MI 24 hours | Baseline | 1.9 MI 2 weeks | Baseline | 1.9 MI 24 hours | Baseline | 1.9 MI 2 weeks |
| 1       | 19       | 26           | 18       | 23           | 16       | 24           |
| 2       | 26       | 28           | 19       | 26           | 17       | 19           | 23       | 25           |
| 3       | 18       | 20           | 14       | 19           | 16       | 17           | 14       | 22           |
| 4       | 18       | 17           | 16       | 22           |          |              |          |               |

Table 2
Clinical Chemistry Results. Raw data of blood urea nitrogen measurements taken from groups exposed to an MI of 1.9 or 1.0 (N = 31). Blood was collected prior to contrast imaging (baseline) and at the final time point of either 4 hours or 2 weeks. Data can also be found in Nyankima et al. [1].

| Subject | High MI | Moderate MI |
|---------|---------|-------------|
|         | Baseline | 1.9 MI 4 hours | Baseline | 1.9 MI 2 weeks | Baseline | 1.0 MI 4 hours | Baseline | 1.0 MI 2 weeks |
| 1       | 21       | 21           | 18       | 21           | 23       | 25           | 27       | 21           |
| 2       | 20       | 24           | 20       | 18           | 24       | 27           | 25       | 25           |
| 3       | 17       | 20           | 15       | 18           | 24       | 25           | 21       | 21           |
| 4       | 17       | 18           | 15       | 18           | 21       | 24           | 21       | 21           |
| 5       | 18       | 18           | 15       | 20           | 24       | 24           |          |               |
| 6       | 17       | 17           | 15       | 18           | 21       | 21           | 17       | 20           |
| 7       | 17       | 20           | 17       | 18           | 21       | 24           | 18       | 20           |
| 8       | 15       | 17           | 18       | 18           | 24       | 25           | 20       |               |

Table 3
Histopathology Results. Counts of total RBCs were collected for groups exposed to an MI of 1.9 or 1.0 (N = 15). Histology was assessed 24 h or 2 weeks after imaging.

| Subject | Kidney Location | High MI | Moderate MI |
|---------|----------------|---------|-------------|
|         |                | 1.9 MI 24 hours | 1.9 MI 2 weeks | 1.0 MI 24 hours | 1.0 MI 2 weeks |
| 1       | R              | 0       | 0           | 0           | 0           |
|         | L              | 0       | 0           | 0           | 0           |
| 2       | R              | 0       | 0           | 0           | 0           |
|         | L              | 0       | 0           | 0           | 0           |
| 3       | R              | 8       | 0           | 0           | 0           |
|         | L              | 27      | 0           | 0           | 0           |
| 4       | R              | 72      | 0           | 0           | 0           |
|         | L              | 0       | 0           | 0           | 0           |
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Conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: E. Chang is supported by a research grant (CG#16013) sponsored by Lantheus Medical Imaging, the distributor of the Definity microbubbles used in this study, though she was not supported during the time of the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104170.

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