Generation of Ultrasound Contrast Bubbles in *In Vivo* Canine Urinary Bladder for Possible Diagnosis of Urinary Reflux

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Abstract-- A less invasive method for diagnosis of urinary reflux is being pursued using high intensity focused ultrasound (HIFU). Microbubbles produced in the urinary bladder, by transcutaneous application of HIFU, can be visualized with a diagnostic ultrasound system and the bubble movement can be tracked from the bladder, through the ureters, and to the kidney, if reflux occurs. Since urine is under-saturated in total gas and filtered by the kidney, it is necessary to increase the gas and/or particle content in the urine in order to produce contrast microbubbles at a minimal level of HIFU. In five canine experiments, sodium bicarbonate (0.0128 M/kg) was infused IV at 3 ml/min for 2 hours with Lasix®. On average for 3 of 5 canines, the infusions resulted in a 63% increase in urine pCO_2 from the baseline (from saline infusion), with mean increase to 87 mmHg. This pCO_2 change decreased the threshold rarefactional pressure for imageable microbubble generation on average to 7.6 MPa (10 s exposure at 1.18 MHz, 25 cycles, 0.5% duty cycle) from 9 MPa (p-value=0.004). The elevated pCO_2 after bicarbonate infusion increased generated echogenicity 16-21 dB higher than for pristine urine or saline infusion for the same peak rarefactional pressure applied (p-value=0.002), and the longevity of the contrast was up to 12.1 seconds. Although these urine property modifications were done with IV administration, eventually urine manipulation should be possible with equivalent bicarbonate and Lasix® doses given orally. Therefore, manipulation of the urine gas content by intake of bicarbonate should be a safe and effective method to assist HIFU in generating copious imageable and residual microbubbles that can potentially be used for less invasive diagnosing urinary reflux.

I. INTRODUCTION

Urinary reflux, or vesicoureteral reflux (VUR), is a condition where urine from the bladder flows retrograde towards the kidney due to a dysfunctional valve action at the junction of the ureter with the bladder, the UVJ. This is a common urinary tract abnormality in children. If urinary tract infection exists, reflux can lead to kidney infection. Furthermore, because of the UVJ patency, the kidney can be exposed to high bladder voiding pressures,. Continued kidney infection or hypertension can result in acute renal dysfunction and/or failure.

The current standard diagnosis method for urinary reflux is vesicoureteral urogram (VCUG). In this procedure the patient is catheterized via the urethra to empty and fill the bladder with X-ray contrast. Since often the patient being evaluated is a young child, this procedure can be particularly uncomfortable and traumatic.

Recently Darge *et al.*, showed that VUR could be diagnosed using a 10% by volume injection of Levovist®. Using VCUG as a reference, the voiding contrast ultrasound method had a sensitivity of 100% and a specificity of 97% in a study of 76 kidney-ureter units [1]. This study demonstrates the feasibility of using contrast enhanced ultrasound imaging, instead of ionizing methods, to diagnose VUR successfully. However, this procedure still requires catheterization of the patient.

A high intensity focused ultrasound (HIFU) system has been developed and used in several previous experiments [2,3]. With this system, past canine experiments have shown the difficulty in generating contrast microbubbles in *in vivo* urine due to naturally low gas and particulate content. An aqueous study using the HIFU system showed the significance of increasing particle content and CO_2 saturation on decreasing bubble generation threshold and increasing echogenicity [2]. Rabbit experiments again indicated the success in contrast generation lies in having particulates as nuclei and higher urine pCO_2 [3]. Rabbits have naturally occurring particulates, unlike humans or canines, so future work would be done with canines since their urine is thought to be more similar to human urine.

This transcutaneous HIFU diagnosis method may minimize or eliminate the need for catheterization and exposure to ionizing radiation. The *in vivo* canine experiments, described below, lead to more reproducible contrast microbubble generation in bladders where the urine was manipulated by intravenous infusion of sodium bicarbonate. This urine manipulation method will aid in the eventual transfer of this non-invasive urinary reflux diagnosis technique to humans.

II. MATERIALS AND METHODS

The HIFU system consists of a 1.18 MHz single-element, spherically focused, transducer (145 mm x 105 mm aperture, 100 mm radius of curvature, **f**1/0.75, with central whole 68 mm diameter, Imasonic S.A., Besançon, France) was used to generate acoustic bursts. High pressure bursts of 25 cycles were generated using a 2.5 kW R.F. gated amplifier (Model 350, Matec Instruments, Inc., Northborough, MA). A 3.75 MHz linear diagnostic ultrasound array (PowerVision 7000, Toshiba America Medical Systems, Tustin, CA) was placed in the center hole of the power transducer. The focal position of the power transducer was identified in the probe image. A S-VHS video recording was made for all scanning, and the video images were used for echogenicity calculations.

Five female canines (various breeds), ≥ 25 kg, were anesthetized with Isoflurane and breathed air. The animal preparation and experimental setup (diagramed below, Figure 1) was similar to past experiments [3].

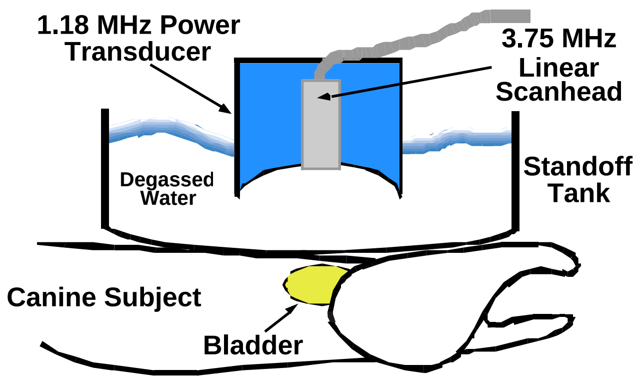

Figure 1. In vivo experimental apparatus.

The transducers were placed in the standoff tank so that the power transducer focus was in the center of the filled bladder. For each canine, the acoustic burst amplitude delivered into the bladder was ramped from 4.9 to 9.2 MPa peak rarefactional. At each amplitude, a 10 second application of 25 cycle bursts at 0.5% duty cycle was delivered. If no bubbles were visualized, then the amplitude was stepped up after waiting 20 seconds. This continued until the threshold for visible contrast generation was achieved. Some super-threshold amplitudes were applied to the urine after waiting 1 minute or more, in order to make echogenicity calculations.

This ramping to determine threshold for imageable contrast generation was done for each canine up to 6 times, twice for each of 3 conditions: (1) pre-intervention, (2) saline infusion, (3) sodium bicarbonate infusion. After the canine was anesthetized, one threshold determination was made on its pristine urine. Next, the canine was catheterized, and another threshold test was done. The urethral catheterization was necessary for urine sample collection throughout the experiment. Care was taken to ensure the inserted end of the catheter was not in the power transducer beam. This second threshold test could indicate if the catheterization effected the threshold by introducing particulates or air. These first two threshold tests on the canine's pristine urine were categorized together as pre-intervention data. If the canine had urinated and emptied its bladder earlier (which was sometimes the case), then the threshold was not tested. After these pre-intervention tests, the bladder was emptied.

The second condition involved infusion of 0.9% saline solution with 0.06 mg/kg/hr Lasix® (Furosemide, Hoechst Roussel Vet, Warren, NJ) at 3 ml/min. The Lasix® was used to ensure that enough urine was produced at a constant rate so that threshold measurements could be made in a bladder that was sufficiently distended. The dosage of Lasix® was determined by using half of the dosage by weight used in a human study on frusemide [4]. An initial 0.12 mg/kg/hr Lasix® bolus was administed in 10 ml of saline to initiate the urine production; the 3 ml/min infusion was continued for about 2 hours. After the first 45 to 60 minutes the bladder was typically full enough, and a threshold measurement was made. Then the bladder was emptied and a second saline threshold test was done after waiting for the bladder to fill again. At the end of the saline infusion condition, the bladder was emptied.

Infusion of 0.0023 mol/kg/hr of sodium bicarbonate (Radix Laboratories, Inc., Eau Claire, WI) in 0.9% saline and 0.06 mg/kg/hr Lasix® at 3 ml/min was the third condition. This dosage of sodium bicarbonate was modeled after a human study that used bicarbonate infusion on healthy volunteers to determine urinary pCO_2 tension and bicarbonate excretion [5]. Half of the human dosage by weight of bicarbonate was used for the canines. Similarly to the saline condition, the bicarbonate was infused over about 2 hours and two threshold measurements were made after each bladder empty and fill cycle.

Urine was collected from the bladder via the urethral catheter. The pH, pCO_2, pO_2, and other fluid values were measured using a blood-gas analyzer (ABL5, Radiometer America Inc., Westlake, OH). After the completion of all threshold tests, the canine was euthanized and the bladder excised. The tissue was stored in formalin for dissection and visual inspection of bioeffects. All animal experiments performed for this research adhere to the national guidelines for the ethical treatment of animals, in keeping with NIH requirements.

The video recorded images at a rate of 6 frames/second were captured from S-VHS for 10 to 20 seconds after insonification at each applied amplitude. To compute echogenicity, the captured images were stacked together (analysis performed using the public domain NIH Image program, available at http://rsb.info.nih.gov/nih-image/) along with two images taken before the insonification (background images for reference). A spatially identical region of interest (ROI), which included the area inside the bladder and excluded the bladder wall and any avoidable reverberations or image artifacts, was selected for this stack of images. For each image ROI a histogram was generated. The mean pixel value and standard deviation of the background histograms were used to threshold all the histograms, and then a background subtraction was performed. After log decompression, the resulting histogram for each image was integrated to obtain a quantitative measure of the increase in echogenicity.

III. Results and Discussion

In all 5 canines, residual imageable contrast microbubbles were generated more consistently after IV infusion of sodium bicarbonate (see example images in Figure 2). The urine pCO_2 increased 63% on average after bicarbonate infusion (see Figure 3). This and further analysis discussed will involve 3 of the 5 canines. Two canines were excluded from the analysis that caused poor image quality due to reverberations resulting from scanning normal and at a long water path from the skin surface. Extra overlying tissue (mostly fat, ≥ 2.5 cm) further deteriorated the image quality.

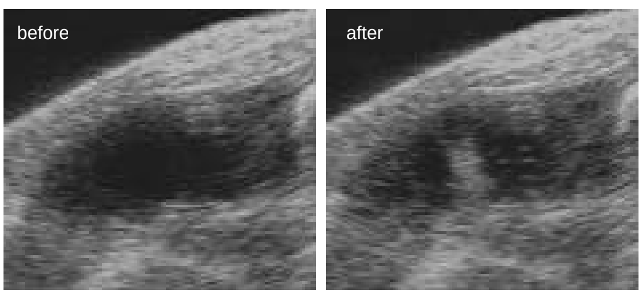

Figure 2. Images before and after insonification at 8.8 MPa after bicarbonate intervention.

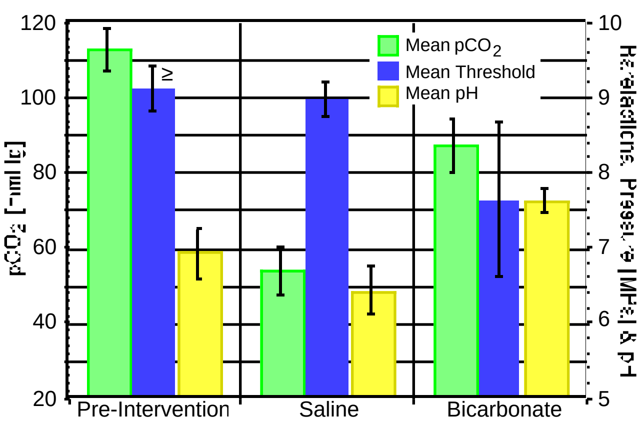

Figure 3. Mean pCO_2, Threshold, and pH for 3 canines.

Even though the pCO_2 in pre-intervention condition was greater than that for bicarbonate infusion, the threshold for contrast generation was higher than for bicarbonate (Figure 3). In many of the pre-intervention condition threshold tests, no bubble generation was observed so the highest pressure applied (9.2 MPa) was assigned as the threshold. Therefore, the pre-intervention threshold plotted is the minimum estimated threshold; it is likely that the actual mean threshold for this condition is greater than 9.1 MPa. The high threshold in the pre-intervention case may perhaps be explained by the lower urine pH, while the combination of the high pCO_2 and high pH in the bicarbonate condition resulted in a lower threshold. So the lower threshold appears to be a function of both elevation in pCO_2 and pH; the bicarbonate infusion achieves both these results. The saline infusion lowers urine pCO_2 and pH, and therefore the threshold is high. The mean threshold for bicarbonate condition in the 3 canines was 7.6 MPa and was the difference in thresholds was significant with p-value of 0.004.

The mean normalized echogenicity (Figure 4) was computed at an applied pressure of 8.57 MPa, which was super-threshold (which was necessary for making the echogenicity calculation) for all bicarbonate conditions. The bicarbonate condition echogenicity was 16 to 21 dB higher than for pristine and saline-conditioned urine (p-value = 0.002). The longevity for the contrast bubbles generated at 8.57 MPa ranged from 9.1 to 12.1 seconds. The larger bubbles generated were determined, by a rise time calculation, to be 10 to 20 µm diameter.

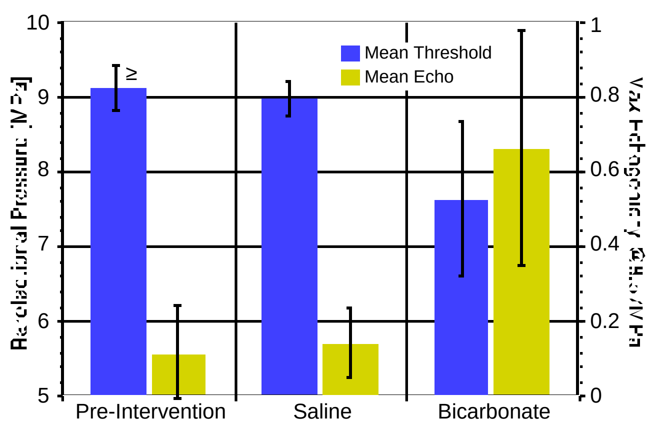

Figure 4. Mean Threshold and normalized echogenicity at 8.57 MPa for 3 canines.

The decreasing linear threshold trend with increasing urine pCO_2, shown in Figure 5, corroborates results of past experiments [2, 3]. A linear fit (solid line) and 95% confidence intervals (dashed curved lines) indicate a correlation between threshold and pCO_2, with r = -0.80.

For saline and bicarbonate conditions at an applied pressure of 8.57 MPa, an exponential trend of increasing echogenicity with increasing urine pCO_2 (Figure 6) exists. Thus, if the urine pCO_2 can be further increased to ≥ 100 mmHg, then there should be reduction in threshold, significant increase in maximum echogenicity, and increase in longevity of the contrast. This was in fact observed in a preliminary canine experiment where infusion of a larger dosage of bicarbonate raised the urine pCO_2 to 230 mmHg. The threshold in this case was 7.65 MPa and the longevity of the contrast bubbles was 85 seconds. Increasing the dosage of the bicarbonate should be safe, since currently the dosage by weight is half that used in humans.

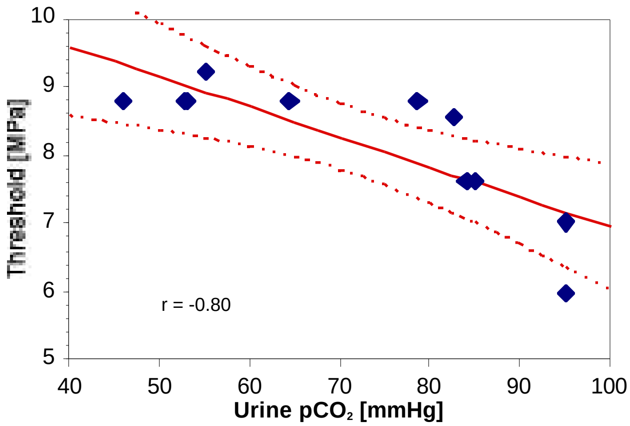

Figure 5. Threshold as a function of urine pCO_2 for 3 canines.

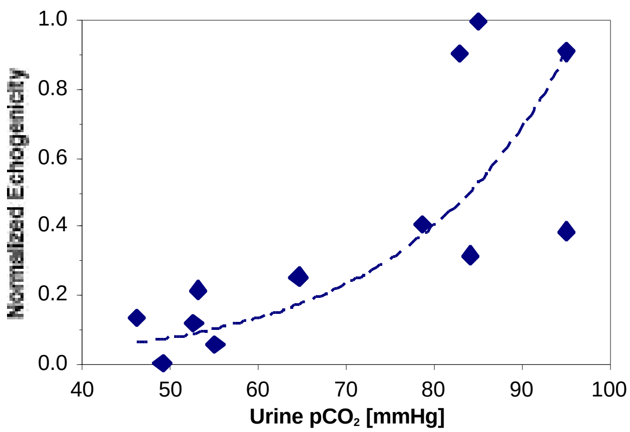

Figure 6. Normalized echogenicity as a function of urine pCO_2 at 8.57 MPa for 3 canines.

By visual inspection of the excised canine bladders, some distortion and possible petechial hemorrhage of the inner lining of the bladder was observed. This was not unexpected considering the large number of insonifications (48 on average) on each of the bladders to determine thresholds for the three conditions. However, in an actual clinical procedure, only a few insonifications will be necessary to generate contrast bubbles; therefore, minimal bioeffects are expected.

Contact scanning could better imaging, and may be done in several ways, such as translating the scanhead down to the skin surface following insonification. Harmonic imaging may also improve image quality.

IV. CONCLUSIONS

The manipulation of the urine carbon dioxide content by infusion of sodium bicarbonate was significant in the lowering the imageable bubble generation threshold and increasing the amount of residual contrast bubbles. Although the manipulation was done by IV infusion in these canine experiments, eventually the procedure will involve oral ingestion of bicarbonate, which is already used in common clinical exams; this will further minimize the invasiveness of this procedure.

With recent advances in development of an acute canine reflux model, experiments implementing this bicarbonate intervention with HIFU contrast bubble generation can be tested. Once the bicarbonate-HIFU contrast generation has been shown to be safe and effective in detecting reflux in the canine model, development of a clinical procedure can begin. This bicarbonate-HIFU technique potentially be a less invasive method for diagnosing urinary reflux by eliminate the need for catheterization and exposure to ionizing radiation.

ACKNOWLEDGMENTS

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