Focused Ultrasound Induced Changes in the Glomerular Ultrafiltration

Ph.D Thesis

Krisztina Fischer, M.D.

Semmelweis University
Doctoral School of Clinical Medicine

Mentors: Dr. András Szabó Professor of Pediatrics
Dr. György Reusz Professor of Pediatrics
Dr. Ferenc Jolesz Professor of Radiology

Official Academic Reviewers: Dr. Tamás Szabó, PhD
Dr. László Wagner, PhD

Ph.D. Theoretical Exam Committee, Head:
Dr. László Rosivall, Professor of Physiology

Ph.D. Theoretical Exam Committee: Dr. Gábor Kovács, Ph.D
Dr. László Szabó Professor of Pediatrics

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1. Introduction

The glomerular barrier has three layers playing important role in the glomerular ultrafiltration. These layers are from the blood to the formative urine: endothelial layer, glomerular basement membrane and the epithelial or podocyte layer. These layers of the glomerular barrier continiously communicate with each other ensuring the momentary glomerular permeability. Although not all of the elements of these communication are well known, there is substantial evidence supporting the existence of the intrapodocyte contractile structure, which can actively change the podocyte's shape and the interpodocyte distance.

Various ultrasound generated acoustic-mechanical effects can induce transient changes in cell permeability and function. These effects can be enhanced with a microbubble-based ultrasound contrast agent.

Ultrasound and microbubbles can also be used to enhance vascular permeability. Low intensity focused ultrasound (FUS) exposures (sonications) combined with the administration of gas microbubble-based diagnostic ultrasound contrast agents, have been shown to temporarily disrupt the blood-brain barrier.

The goals of my PhD work were: to test the acoustic sensitivity of the glomerular barrier, to examine the FUS effects on the tubular function and furthermore, with the use of ultrastructural studies, to find the responsible mechanism for the observed changes in the glomerular ultrafiltration. Our results may help the better

understanding of the glomerular function and with further research they can serve as basics for new therapeutic or diagnostic design development.

2. Aims of our study

2.1 Focused ultrasound's effects on the glomerular ultrafiltration in the presence of microbubble based contrast agent

Our first aim was to demostrate the effect of FUS on the glomerular ultrafiltration and permselectivity.

2.1.1 Is there any change in the small and/or the large molecule clearance following the FUS treatment?

2.1.2 Is there any change in the tubular function following the FUS treatment?

2.1.3 Is there any structural alteration following the FUS treatment?

2.2 Are the observed changes in the glomerular ultrafiltration dependent of the applied ultrasound power level?

Several data support the FUS treatment power dependent effects on the blood-brain barrier (BBB) disruption. The higher the ultrasound power the higher the probability of the BBB opening and the larger the amount of molecules go through the BBB.

These results arose the question that the FUS treatment's effects on the kidney function may be power depentent alike. Our second aim

was to demostrate the FUS treatment's power dependent effects on the glomerular ultrafiltration.

2.3 Our third aim was to demonstrate the physical effects of the FUS treatment on the glomerular barrier.

While the exact mechanism for the biological membran's permeability enhancement is unknown, it is supported by several publications that the sonications increase both passive and active transport mechanisms as well as induce physiological changes There are numerous results supporting that the endothelial cells continuity is loosen or disrupted following the FUS treatment. Using ultrastructural examination we looked for the potential alterations in the glomerular barrier's physical properties following the FUS treatment.

3. Methods

3.1.1 The focused ultrasound's effects on the glomerular permeability

In these experiments we treated the exposed left kidney in 17 healthy rabbit with FUS combined with an ultrasound contrast agent. The right kidney served as a control. Three acoustic power levels were applied: 0.4 W (in six rabbits), 0.9 W (in six rabbits) and 1.7 W (in five rabbits). Three animals not treated with FUS served as controls. One of these received ultrasound contrast agent. We applied 15 sonications in theese experiments.

Animal preparation

The rabbits were anesthetized with a mixture of xylazine and ketamine Saline infusion was started after the induction of the anesthesia. The left kidney was surgically exteriorized to allow easy targeting by the acoustic beam. Its urine was collected from the bladder using a Foley catheter placed in the urethra. The right kidney's ureter was cut and a ureter catheter was inserted to collect its urine. Blood pressure was continuously monitored during the procedure using a Grass polygraph via a cannula placed in the carotid artery. A catheter for intravenous injection was placed in the left ear vein.

3.1.2 Power dependent changes in the glomerular ultrafiltration following the focused ultrasound treatment with administration of the ultrasonographic contrast agent.

In these experiments we treated the exposed left kidney in 16 healthy rabbit with FUS combined with an ultrasound contrast agent. The right kidney served as a control. Three acoustic power levels were applied: 0.4 W (in five rabbits), 0.9 W (in six rabbits) and 1.7 W (in five rabbits). Four animals not treated with FUS served as controls. We applied 30 sonications in these experiments.

3.2.1 The timeline of the experiments

3.2.1.1 The focused ultrasound treatment's effects on the small and/or large molecule clearance

The animal preparation was approximately 90 minutes. The animals were placed on the experimental set up 30 minutes before the measurements started. Urine samples were collected in 15 minutes fractions, we collected 13 urine samples during the experiment. In the midpoint of every urine collection blood sample was collected from the carotid catheter.

3.2.1.2 Power dependent changes in the glomerular ultrafiltration following the focused ultrasound treatment with administration of the ultrasonographic contrast agent.

In these experiments, urine samples were collected in 20 minutes fractions. In the midpoint of every urine collection blood sample was

collected from the carotid catheter. The first measurement served as the baseline measurement. The FUS treatment lasted for 60 minutes.

3.2.2 The experimental set up

The FUS transducer was housed in a manually-operated mechanical positioning system and submerged in a tank of degassed, deionized water. The animal lay on its side on a plastic tray with a 3×5 cm rectangular hole cut in it that was mounted on the top of the tank. A thin plastic sheet was loosely attached to the top of the tray and pushed through the rectangular hole to form a bag that was filled with degassed water (monitored and maintained at ~37°C by a heating coil). The exteriorized left kidney hung in the water bag. The bottom of the water bag rested on a taut acoustically transparent plastic membrane that was mounted below it.

3.2.3 The focused ultrasound

3.2.3.1 The transducer

The acoustic fields were generated with an air-backed spherically curved transducer (frequency: 260 kHz; diameter/radius of curvature: 10/8cm) that was manufactured in-house.

3.2.3.2 The sonications - The focused ultrasound treatment's effects on the small and/or large molecule clearance

Each sonication consisted of thirty 10 ms pulses at a repetition frequency of 1 Hz. The targets were located approximately one cm deep into the kidney in a single plane. Fifteen targets were sonicated

at one cm intervals to cover the extent of the 3×5 cm rectangular hole through which the kidney was hanging.

3.2.3.3 The sonications - Power dependent changes in the glomerular ultrafiltration following the focused ultrasound treatment with administration of the ultrasonographic contrast agent.

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3.2.4 The microbubble based ultrasonografic contrast agent

A bolus of ultrasound contrast agent was injected intravenously at the start of each of the sonications at a dosage of 10 μl/kg.

3.2.5 Evaluation of the kidney function

3.2.6.1 Blood and urine collection and analysis

The collected urine and blood samples were centrifuged and the supernatant were used for fluorescent and regular laboratory analysis.

3.2.6.2 Glomerular permselectivity measurements

A mixture of fluorescent dextran solution, containing 3,000 Da and 70,000 Da dextran, was injected the animals twice during the experiments. The first injection was administered after the first urine sample collection. The second injection was administered after the sonications ended.

3.2.6.3 Regular laboratory measurements

The urine and plasma creatinine, sodium, phosphate and the urine protein concentration, gamma-glutamyltrasferase (GGT) activity were measured using rutin laboratory methods.

3.2.6.4 The calculation of the glomerular filtration rate

The creatinine clearance calculation was used to estimate the glomerular filtration rate.

3.2.6.5 The proteinuria and estimation of the tubular function

The protein-to-creatinine ratio, and the GGT-to-creatinine ratio were calculated in each urine fraction. The fractional sodium excretion (FENa%), and the tubular phosphate reabsorption were also calculated.

3.2.7 Histological examination

All of the treated and control kidneys were evaluated for histological abnormalities using Haematoxylin and Eosin (H&E) and Periodic

Acid Schiff (PAS) staining. The tissue (glomerular and tubular) damage was evaluated.

3.2.8 Ultrastructural examination

Ultrathin sections were made for transmission electronmicroscopy evaluation. The podocyte distances were measured on the electromicrophotographs using AMT image software.

3.2.9 Statistical analysis

In each anaimal, in every measurement the ratio of the left (treated) and the right (control) functional parameter was detremnined. Further on these ratios were labelled as reative dextran, creatinine clearance, urine flow rate, etc.

The Kolmogorov-Smirnov test was used to determine if the measures were normally distributed. To determine whether the observed enhancement in examined clearances was significant, paired t-tests were performed. Differences were considered significant when $P<0.05$. To determine whether the differences in the podocyte distances were significant two-tailed t-tests were performed. Differences were considered significant when $P<0.05$.

SPSS 17.0, a statistical analysis software, was used for the statistical analysis.

4. Results

4.1 The glomerular permeability and ultrafiltration

4.1.1 The glomerular permeability

During and immediately after the FUS treatment we found 1.54-; 1.56-; and 1.70-fold increase in the relative dextran 70,000 Da clearance (0.4; 0.9 and 1.7 W ultrasound power group accordingly). This increase in the relative dextran 70,000 Da clearance was accompanied with 1.41-; 1.43-; and 1,63-fold increase in the relative urine flow rate. These ratios were significantly larger ($p<0.05$) than the pretreatment values. After treatment, the relative dextran 70,000 Da and urine flow rate ratios were not significantly different than the pretreatment values.

4.1.2 The glomerular ultrafiltration

The overall enhancement (without the distinction of the ultrasound power treatment groups) both in relative creatinine and dextran 3,000 Da clearances were 1.23 fold. The overall enhancement was significant for both the relative creatinine and dextran 3,000 Da clearances. After treatment, the relative creatinine and dextran 3,000 Da clearances were not significantly different than the pretreatment values.

4.2 The proteinuria and the tubular function

4.2.1 The proteinuria

The FUS treatment at 0.4 W did not result in an elevated protein-to-creatinine ratio. It was significantly elevated at 0.9 W, but remained within the normal range. At the highest power level (1.7 W) this ratio exceeded the normal range (the elevation was significant). This elevated protein-to-creatinin ratio returned back to normal range after 45 minutes after the treatment ended.

4.2.2 The tubular function

The tubular function was followed through the relative FENa%, TRP% and urinary GGT-to-creatinine. Notable change was found only in the relative FENa% at the two higher power level (1.27-; and 1.24-fold according to the power levels, $p<0.05$). The FENa% returned back to pretreatment level 30 minutes after the treatment ended.

4.3 Histological examination

No anatomic damage was observed in the HE stained sections at the two lower power levels (0.4 W and 0.9 W). Minor tubular hemorrhages appeared after sonication at 1.7 W. No interstial hemorrhage was seen at any power level. The PAS staining showed intact proximal tubular brush borders and normal tubular structure at every examined power level.

4.4 Acoustic emission

The „bubble activity" that may accompany the sonications can be detected with a cavitation detector. In many of the sonications this „bubble activity" was observed. This activity appeared as a large increase in the spectral energy at and around the resonant frequency of our passive cavitation detector. Emission at the harmonics of the ultrasound frequency was observed as well as subharmonic and ultraharmonic emission at ½ and 3/2 the ultrasound frequency. Such activity was not observed when the sonication location was in water only.

4.5 The relationship between the relativ creatinine clearance and the applied acoustic energy

The second set of experiments that included 30 sonications we found 1.06-; 1.34-; and 1.88-fold increase in the average relative creatinine clearance (0.4 W; 0.9 W and 1.7 W respectively). These increases were significant ($p<0.05$) at the two higher power level (0.9 W and 1.7 W). The observed increase in the creatinine clearance was dependent of the applied acoustic power level ($R^2=0.998$).

4.6 Proteinuria and tubular function

The FUS treatment resulted significant ($p<0.05$) elevation in the relative protein-to-creatinin ratio at every acoustic power level. This elevation exceeded the normal range at the highest acoustic power level (1.7 W). No significant change was observed in the tubular function.

4.7 The ultrastructural examination

We found significant, power dependent increase (1.63; 1.66 and 1.72, $p<0.01$) in the podocyte distance at each acoustic power level in the animals that showed increase in the creatinine clearance during the FUS treatment. We did not find alteration in the podocyte distance in the animals that did not respond with creatinine clearance increase during the FUS treatment.

5. Conclusion

1. In healthy kidney the creatinine clearance and the dextran 3,000 Da clearance can be increased with focused ultrasound in the presence of microbubble based ultrasonographic contrast agent.

2. In healthy kidney the dextran 70,000 Da clearance (which normally hardly cleared by the kidney) and urine flow rate can be increased with focused ultrasound in the presence of microbubble based ultrasonographic contrast agent.

3. The FUS treatment resulted transient increase in the protein-to-creatinine ratio. This increase in the proteinuria remained in the normal range when the two lower power level was applied.

4. The FUS did not result major alteration in the tubular function.

5. Based on the light microscopy examination the FUS treatment did not result structural alteration in either the cortical or the medullar part of the rabbit kidney.

6. Based on the ultrastructural examination the FUS treatment resulted increase in the podocyte distance, which may be responsible for the observed changes in the glomerular permeability and ultrafiltration.

7. The higher number of sonications and the introduction of the imaging ultrasound resulted more significant change in the glomerular ultrafiltration.

8. In healthy kidney focused ultrasound in the presence of microbubble based ultrasonographic contrast agent resulted power dependent changes in the glomerular ultrafiltration.

Publications related to the thesis

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