The Effects of Modulating Endothelial Nitric Oxide Synthase (eNOS) Activity and Coupling in Extracorporeal Shock Wave Lithotripsy (ESWL)

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Introduction

ESWL is a clinical therapy to break down kidney and uretal stones into smaller fragments that are more easily eliminated through the urinary tract. High-energy shock waves are focused on the stone to cause shear stress and cavitation bubbles which synergistically ablate the stones. While ESWL is the preferred treatment for kidney stones over invasive surgeries, the repetitive shock waves necessary to break up the stones may also cause damage to the renal vasculature endothelium and that can lead to chronic hypertension [1]. Previous studies have found that ESWL can cause endothelial dysfunction which is characterized decreased nitric oxide (NO) bioavailability and increased production of reactive oxygen species (ROS) such as superoxide (O_2^-) [2]. Normally, endothelial nitric oxide synthase (eNOS) is in a coupled state which forms NO in the presence of essential cofactor tetrahydrobiopterin (BH_4) and molecular oxygen. Oxidative stress, such as that caused by ESWL-induced ROS, can cause BH_4 to be oxidized to dihydrobiopterin (BH_2). When the BH_2:BH_4 ratio is increased, eNOS becomes uncoupled and produces O_2^- instead of NO [2, 3] (Figure 1). O_2^- is short-lived and converted to hydrogen peroxide (H_2O_2) in blood by superoxide dismutase. Protein kinase C epsilon (PKCε) has previously been found to regulate eNOS activity via phosphorylation at serine-1177. Cell-permeable PKCε peptide activator (PKCε+) increases eNOS activity while PKCε inhibitor (PKCε-) reduces eNOS activity [2]. Using a combination of eNOS cofactors BH_4 or BH_2 with eNOS activity regulators PKCε+ or PKCε-, we can explore the role of modulating eNOS to reduce oxidative stress and endothelial dysfunction caused by ESWL.

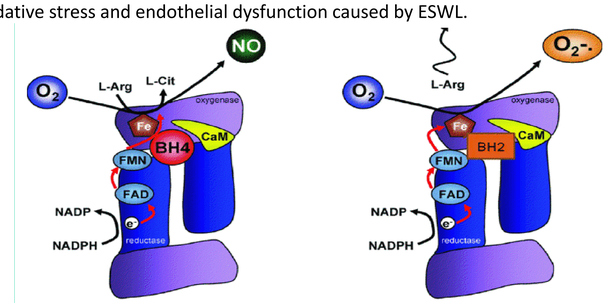

Figure 1. Coupled eNOS and Uncoupled eNOS. BH_4 is the cofactor of coupled eNOS which results in NO production. ESWL-induced oxidative stress results in an increased BH_2:BH_4 ratio. BH_2 is the cofactor for uncoupled eNOS which produces O_2^- and subsequently H_2O_2.

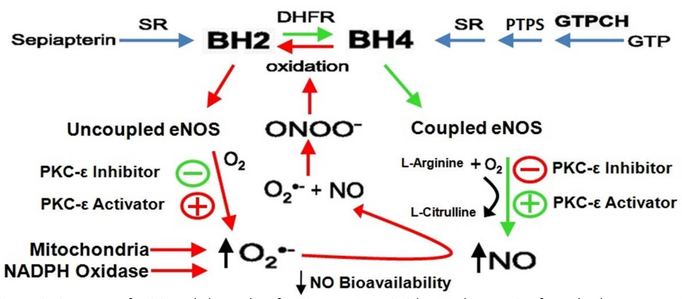

Figure 2. Sources of ROS and the Role of PKCε. NADPH Oxidase releases O_2^- from leukocytes and endothelial cells. Mitochondria releases O_2^- via incomplete oxidative phosphorylation. Uncoupled eNOS releases O_2^- in the presence of BH_2. Increased O_2^- quenches NO produced from coupled eNOS to produce ROS such as peroxynitrite ($ONOO^-$) which reduces NO bioavailability and further increases oxidative stress. PKCε+ increases coupled and uncoupled eNOS activity, and PKCε- decreases coupled and uncoupled eNOS.

Hypothesis

We hypothesize that ESWL treatment will decrease NO and increase H_2O_2 release in rat renal veins compared to no-ESWL controls. We further hypothesize that a post-ESWL i.v. bolus of PKCε+/BH_4 will increase NO and decrease H_2O_2 release compared to ESWL + saline controls. Whereas, we expect a post-ESWL i.v. infusion of PKCε+/BH_2 will decrease NO and increase H_2O_2 compared to ESWL + saline controls. We hypothesize that PKCε- given with either BH_4 or BH_2 after ESWL will increase NO and decrease H_2O_2 release compared to ESWL + saline controls.

Methods

Male Sprague-Dawley rats (275-325g, Charles River, Springfield, MA) were anesthetized with an induction dose of sodium pentobarbital (60 mg/kg) intraperitoneally. A maintenance dose (30 mg/kg) was administered at intervals of 45 minutes unless otherwise required. The rat also received sodium heparin (1000 USP units/mL) i.p. to act as an anticoagulant. The rat's left external jugular vein was exposed and catherized with a 24-gauge angio-catheter for drug or saline infusion post-ESWL treatment. The rat was then subjected to a mid-line laparotomy, and the left renal vein was isolated. A 22-gauge angio-catheter was inserted into the left renal vein in direct opposition of the blood flow. A NO or H_2O_2 microsensor was inserted into the renal vein catheter and connected to the TBR 4100 Free Radical Analyzer (World Precision Instruments, Inc., Sarasota, FL) which produces a trace showing real-time measurements of NO or H_2O_2 release recorded in picoamps (pA). The microsensors receive an electrical signal proportional to the free radical concentration through an oxidation/reduction reaction. Baseline measurements were then taken until a stable baseline (i.e., 300 pA decrease per 300 seconds) was achieved. Thereafter, the baseline was set to a "zero" reading, and all measurements post-ESWL were expressed as relative change from baseline. Once a stable baseline was established, ESWL treatment was initiated by a Dornier Epos Ultra Lithotripter (1000 shocks, 500 at 60 beats/min, 500 at 120 beats/min, 16kV, 1.3mHz). To simulate conditions in the no-ESWL control group, the approximate time of treatment (13 min) was maintained without ESWL treatment. Immediately following ESWL or at the same time for no-ESWL controls, 0.5 mL of saline or drug bolus was infused through the jugular vein canulation followed by a 0.5mL saline flush. Experimental groups included combinations of PKCε+ (N-Myr-HADPIGYD, 1097 g/mol, Genemed Synthesis) or PKCε- (N-Myr-EAVSLKPT, 1054 g/mol, Genemed Synthesis) with BH_4 (314 g/mol, Cayman Chemicals) or BH_2 (239 g/mol, Cayman Chemicals). Recordings of NO and H_2O_2 release were taken throughout the experiment (baseline, ESWL end, 30 min post-ESWL). The microsensors were calibrated prior to each experiment in order to create a standard calibration curve via a stepwise dose-response to the appropriate standard solution. NO readings in pA were converted to nanomoles/L (nM), and H_2O_2 readings in pA were converted to micromoles/L (μM).

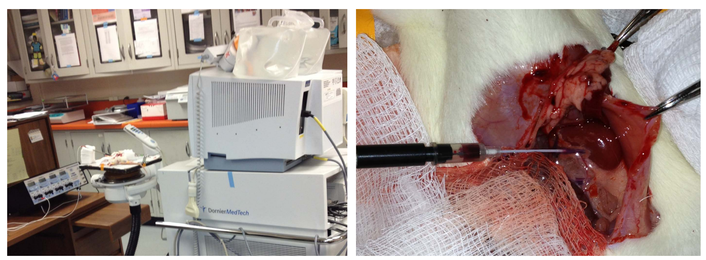

Figure 2. The rat was placed supine on a working board with the left kidney in the focal point of the lithotripter. The microsensor was supported by a catheter in the left renal vein, and the microsensor tip was positioned so that it was in direct contact with the renal vein blood flow.

Experimental Groups:

Nitric Oxide (NO)

1. No-ESWL with Saline (Control) (n=6)
2. ESWL with Saline (Control) (n=5)
3. ESWL with PKCε+/BH_4 (n=5) (0.9 mg/kg; 0.8 mg/kg)
4. ESWL with PKCε+/BH_2 (n=5) (0.9 mg/kg; 2 mg/kg)
5. ESWL with PKCε-/BH_4 (n=5) (0.8 mg/kg; 0.8 mg/kg)
6. ESWL with PKCε-/BH_2 (n=5) (0.8 mg/kg; 2 mg/kg)

Hydrogen Peroxide (H_2O_2)

7. No-ESWL with Saline (Control) (n=6)
8. ESWL with Saline (Control) (n=6)
9. ESWL with PKCε+/BH_4 (n=5) (0.9 mg/kg; 0.8 mg/kg)
10. ESWL with PKCε+/BH_2 (n=5) (0.9 mg/kg; 2 mg/kg)
11. ESWL with PKCε-/BH_4 (n=5) (0.8 mg/kg; 0.8 mg/kg)
12. ESWL with PKCε-/BH_2 (n=5) (0.8 mg/kg; 2 mg/kg)

All data was presented as means ±SEM. The data for each time-point were compared by ANOVA using post-hoc analysis with the Student Newman Keuls test. Probability values of less than 0.05 were considered to be statistically significant.

Results

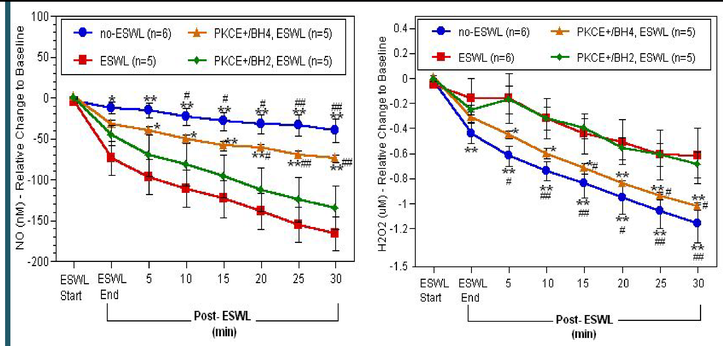

Figure 3. Effect of PKCε+ Combined with BH_4 or BH_2 on Real-Time Blood NO and H_2O_2 Release after ESWL. ESWL significantly decreased NO release and increased H_2O_2 release (5-30 mins post-ESWL) compared to no-ESWL controls. Administration of PKCε+/BH_4 after ESWL was similar to no-ESWL controls, significantly increasing NO and decreasing H_2O_2release (5-30 mins post-ESWL) compared to ESWL controls. Post-ESWL infusion of PKCε+/BH_2 was similar to the ESWL control group in both NO and H_2O_2 release. (*p≤0.05, **p≤0.01, compared to ESWL controls) (#p≤0.05, ##p≤0.01, compared to ESWL with PKCε+/BH2)

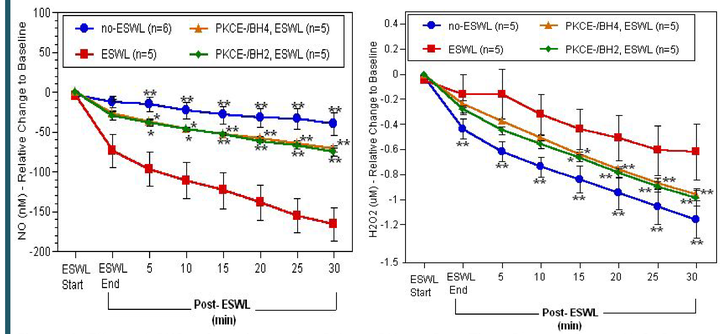

Figure 4. Effect of PKCε- Combined with BH_4 or BH_2 on Real-Time Blood NO and H_2O_2 Release after ESWL. Post-ESWL infusions of PKCε-/BH_4 or PKCε-/BH_2 are both similar to no-ESWL controls, significantly increasing NO release (5-30 mins) and decreasing H_2O_2 release (15-30 mins) compared to ESWL controls. (*p≤0.05, **p≤0.01, compared to ESWL controls)

Conclusions

ESWL treatment decreased NO and increased H_2O_2 blood levels compared to no-ESWL controls. This supports our hypothesis and previous findings in this lab that ESWL causes oxidative stress and reduced NO bioavailability. Post-ESWL PKCε+/BH_4 significantly attenuated the adverse effects of ESWL by increasing NO and decreasing H_2O_2 release compared to ESWL+saline. This suggests that this combination enhances eNOS in its coupled state. Whereas, post-ESWL PKCε+/BH_2 was similar to ESWL control in H_2O_2 and NO, suggesting that BH_2 is nearing saturation to the eNOS binding site. In contrast, post-ESWL PKCε- with either BH_4 or BH_2 resulted in increased NO and decreased H_2O_2 compared to ESWL+saline. This suggests that PKCε- attenuates eNOS uncoupled activity after ESWL. Potentially, this study can help to develop therapeutic uses for PKCε+/BH_4 or PKCε- in the attenuation of vascular endothelial dysfunction following ESWL treatment and possibly eliminate or reduce the acute renal complications that may lead to chronic conditions such as hypertension.

References

1. McAteer JA and Evan AP. (2008) The Acute and Long-Term Effects of Shock Wave Lithotripsy. *Seminars in Nephrology, 28(2)*, 200-213.
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3. Chen, Q., Kim, E. E. J., Elio, K., Zambrano, C., Krass, S., Teng, J. C., et al. (2010) The role of tetrahydrobiopterin and dihydrobipterin in ischemia/reperfusion injury when given at reperfusion. *Advances in Pharmacological Sciences, 2010*, 1-11.