**Introduction**

ESWL is a clinical therapy to break down kidney and ureteral 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 ($O_2^-$). 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$_4$/BH$_2$ 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$_2$O$_2$) in blood by superoxide dismutase. H$_2$O$_2$ that has previously been found to oxidize eNOS activity via phosphorylation at serine-1177. Cell-permeable PKC epsilon activator (PKCε+) increases eNOS activity while PKC 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.

**Hypothesis**

We hypothesize that ESWL treatment will decrease NO and increase H$_2$O$_2$ release in rat renal veins compared to no-ESWL controls. We further hypothesize that a post-ESWL infusion of PKCε+/BH$_4$ will increase NO and decrease H$_2$O$_2$ 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$_2$O$_2$. Combined with our previous findings in this lab that ESWL causes oxidative stress and reduced NO bioavailability, post-ESWL PKCε+/BH$_4$ treatment will be necessary to attenuate the adverse effects caused by ESWL.

**Methods**

**Figure 1. Coupled eNOS and Uncoupled eNOS.**

In Extracorporeal Shock Wave Lithotripsy (ESWL), the microsensor was inserted into the left renal vein in direct opposition of the blood flow. A NO or H$_2$O$_2$ microsensor was inserted into the renal vein catheter and connected to the TBR 4100 Free Radical Analyzer (World Precision Instruments, Inc., Sarasota, FL) which uses a trace showing real-time measurements of NO or H$_2$O$_2$ release recorded in picomamps (pA). The microsensor receives an electrical signal proportional to the free radical concentration through an oxidation/reduction reaction. Base measurements were then taken until a stable baseline (i.e., 300 pA decrease per 300 seconds) was achieved. 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, 16V, 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 catheter followed by a 0.5mL saline flush. Experimental groups included combinations of PKCε+ (N-Myr-HADPYPHDG, 1097 g/mol, Genemed Synthesis) or PKCε− (N-Myr-EAWSKKT, 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$_2$O$_2$ release were taken throughout the experiment (baseline, end, 30 min after ESWL). The microsensors were calibrated prior to each experiment in order to generate a standard calibration curve. NO measurements were normalized to baseline H$_2$O$_2$ readings in pA were converted to nanomoles/L (nM), reflecting the approximate absorption of light.

**Figure 2. Sources of ROS and the Role of PKCε.** NAPDH 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 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.

**Results**

**Figure 3. Effect of PKCε+ Combined with BH$_4$ or BH$_2$ on Real-Time Blood NO and H$_2$O$_2$ Release after ESWL.** The NO and H$_2$O$_2$ levels were similar to ESWL controls. NO release compared to no-ESWL controls. Post-ESWL infusion of PKCε+BH$_2$ was similar to the ESWL control group in both NO and H$_2$O$_2$ release. (*p≤0.05, **p≤0.01, compared to ESWL controls) (Ap0.05, Ap0.01, compared to ESWL controls with PKCε+BH$_2$).

ESWL treatment decreased NO and increased H$_2$O$_2$ blood levels compared to no-ESWL controls. This supports our hypotheses and previous studies 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$_2$O$_2$ 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 controls. PKCε− attenuates eNOS uncoupled activity after ESWL. Potentially, this study can help to develop therapeutic uses for PKCε+BH$_2$ 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.

**Conclusions**


2. James, E. S., Perkins, K., Chen, Q., Young, L. The role of protein kinase C epsilon in the regulation of endothelial nitric oxide synthase (eNOS) during oxidative stress caused by extracorporeal shock wave lithotripsy (ESWL). 22nd Am PhysioL Symp. 2011, 278-279.