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 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$_2^-$) [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).

Methods

Nitric Oxide (NO) and Hydrogen Peroxide (H$_2$O$_2$) release was measured in the presence of BH$_4$ (0.8 mg/kg) or BH$_2$ (0.8 mg/kg) compared to ESWL + saline control. The microsensor tip was positioned so that it was in direct contact with the renal blood vein flow. H$_2$O$_2$ release was measured with an amperometric cell-permeable substrate (ABT, Genemed Scientific, Inc.) that produces a trace showing real-time measurements of NO or H$_2$O$_2$ release in picoamperes (pA). The microsensor receives an electrical signal proportional to the free radical concentration through an oxidation/reduction reaction. Baseline measurements were 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 shock, 500 at 60 beats/min, 500 at 120 beats/min, 168V, 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 0.5 mL saline flush. Experimental groups included combinations of PKCε+/BH$_4$ (N-Myr-HADPIGYD, 1097 g/mol, Genemed Seminars in Nephrology, 200-213), 1054 g/mol, Genemed Seminars in Nephrology, 200-213) with BH$_4$ (314 g/mol, Genemed Seminars in Nephrology, 200-213) or BH$_2$ (239 g/mol, Genemed Seminars in Nephrology, 200-213). Recordings of NO and H$_2$O$_2$ release were taken throughout the experiment (baseline, 30 min post-ESWL). The microsensor recordings were calibrated prior to each experiment in order to create a standard calibration curve with a stepwise dose-response to the appropriate standard solution. NO readings in pA were converted to nanomoles/L (nM), and H$_2$O$_2$ readings in pA were converted to micromoles/L (µM).

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. PKCε+ ESWL significantly decreased NO and increased H$_2$O$_2$ 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 increased NO release and decreased H$_2$O$_2$ release (5-30 mins post-ESWL) compared to ESWL controls. Post-ESWL infusion of PKCε+/BH$_4$ 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) (P<0.05, **P<0.01, compared to ESWL controls).

Figure 4. Effect of PKCε+ Combined with BH$_4$ or BH$_2$ on Real-Time Blood NO and H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$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 control in NO and H$_2$O$_2$, 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$_2$O$_2$ 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

2. Iames, 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 Urology Symposium, 2011, 278-279.