The Effects of Dihydrobiopterin and Tetrahydrobiopterin on Hydrogen Peroxide and Nitric Oxide Release During Extracorporeal Shockwave Lithotripsy

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Introduction

Extracorporeal shockwave lithrotripsy (ESWL) is an effective, non-invasive clinical therapy utilized to break up stones in the kidney and urinary tract. A lithotripter generates high-energy acoustic pulses and propagates those shock waves through a lens on a region that focuses on the location of the stone, in turn breaking up the stone. The successive pulses generate shearing forces and cavitation bubbles. Cavitation bubbles are the formation and implosion of liquid free zones. The cavitation bubbles implode rapidly to create their own shockwaves that also put pressure on the stone. After treatment, fragmentation of the stone allows the debris to be cleared by the flow of the urinary tract. The problem is that to break up the kidney stone, it requires many repetitive shock waves that not only hit the kidney stone but also the surrounding tissue. Although lithotripsy provides a safer alternative to invasive treatments for removing harmful stones, ESWL may cause prolonged vasoconstriction after ESWL treatment, reducing renal blood flow, and subsequent endothelial dysfunction, which may cause kidney damage leading to acute to chronic hypertension clinically. ESWL-induced vascular oxidative stress and further endothelial dysfunction may be mediated by reduced levels of endothelial-derived nitric oxide (NO) and/or increased reactive oxygen species. Previous studies have shown that ESWL can induce oxidative stress, which can cause an increase in blood hydrogen peroxide (H_2O_2) and a decrease in endothelial-derived NO bioavailability release. Under normal conditions, tetrahydrobiopterin (BH_4) is the cofactor to promote eNOS coupling, and endothelial-derived NO is produced. When the dihydrobiopterin (BH_2) to tetrahydrobiopterin (BH_4) ratio is increased during oxidative stress, such as ESWL, BH_2 promotes eNOS uncoupling and produces superoxide (SO) instead of NO. (1,2) (Figure 1) SO is then later converted to H_2O_2 by superoxide dismutase. BH_4 and BH_2 bind to eNOS with equal affinity, therefore the ratio will determine whether eNOS principally produces NO or SO.

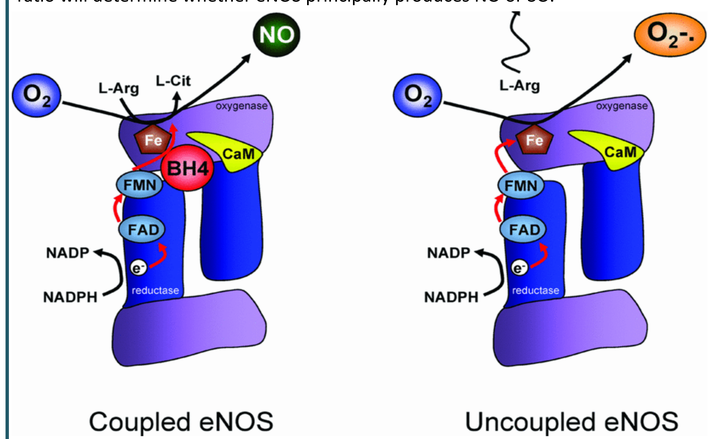

Figure 1. With BH_4 cofactor, coupled eNOS produces NO. During oxidative stress, BH_4 is oxidized to BH_2, and the BH_2 to BH_4 ratio is increased. With BH_2 as the cofactor, eNOS becomes uncoupled. Unable to shuttle electrons to use L-Arginine as a substrate, uncoupled eNOS transfers the electrons to produce SO. Later, SO is converted to hydrogen peroxide by SOD. Adapted from Chen et al. 2010 (1.)

Hypothesis

We hypothesize that the introduction of ESWL will decrease NO release in left renal veins compared to controls receiving no ESWL. Whereas, an increase in H_2O_2 release is expected in the ESWL + Saline group compared to the non-ESWL group. When tetrahydrobiopterin (BH_4)(mol. wt. 241.25)(Cayman Chemicals) is given at the end of ESWL treatment we predict a decrease in H_2O_2 release and an increase in NO release compared to ESWL + saline group. On the contrary, when dihydrobiopterin (BH_2)(mol. wt. 239.23)(Cayman Chemicals) is given at the end of ESWL treatment we predict an increase in H_2O_2 release and decrease in NO release compared to ESWL + Saline group.

Methods

Male Sprague-Dawley rats (275-325 grams, Ace Animals, Boyertown, PA) were anesthetized using sodium pentobarbital with an induction dose of 60mg/kg via intraperitoneal injection. A maintenance dose (30mg/kg) was given at intervals of approximately 45 minutes. The rat was then injected via intraperitoenal injection with 1mL sodium heparin (1000 USP units/mL) to prevent blood clotting. A 24-gauge catheter was inserted into the external jugular vein for drug or saline infusion immediately following ESWL treatment. A mid-line abdominal incision was performed and the left renal vein was exposed. Upon catheterization of the left renal vein with a 22-gauge catheter, the NO or H_2O_2 microsensor (World Precision Instruments, Inc., Sarasota, FL) was inserted through the catheter and connected to the Apollo 4000 Free Radical Analyzer. (World Precision Instruments, Inc.) The trace was recorded until a decrease of one picoamp per second, indicating a stable baseline. After establishment of a stable baseline, ESWL treatment was induced by a Dornier Epos Ultra HE (high-energy) lithotripter (Figure 2). ESWL treatment consisted of approximately 13 minutes of shockwaves, a total of 1000 shocks in two periods of 16kV intensity were transmitted. The first 500 shocks were given at 60 beats per minute followed by 500 shocks at 120 beats per minute. Immediately post-ESWL treatment, 0.5 mL saline or drug bolus was infused through the jugular vein canulation followed by 0.5 mL of saline as a flush. Recordings were taken at the beginning and end of ESWL treatment, then in five minute intervals for 30 minutes post-ESWL treatment.

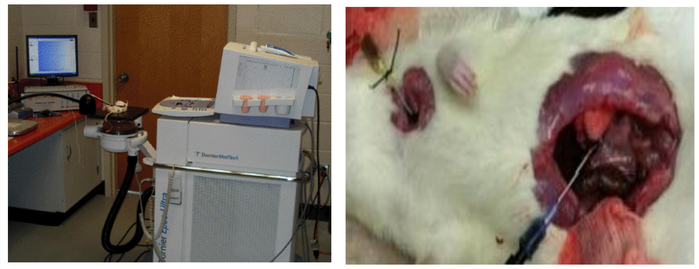

Figure 2. The rat is placed on a board that maintains the left kidney within the focal point of the shock wave transmitter on the lithotripter's mechanical arm. The microsenor is inserted into the left renal vein and supported by gauze in direct opposition to the renal vein blood flow.

A technique was developed to measure renal blood NO and H_2O_2 in real-time using microsensors. Molar concentration of free radicals could not be measured directly *in* vivo, therefore, microsensors, which receive an electrical signal proportional to the concentration of the free radical through a oxidation/reduction reaction, were used to measure NO and H_2O_2 levels. The free radical analyzer collected data in picoamps (pA). Each microsensor was calibrated before each experiment to calculate a standard calibration curve. The standard calibration curve was generated by a stepwise dose-response of the microsensor to the appropriate standard solution.

Experimental Groups:

Nitric Oxide (NO)

- Group 1: No-ESWL, Saline infusion (Control) (n=6)
- Group 2: ESWL, Saline infusion (Control) (n=5)
- Group 3: ESWL + BH_4 6.5 mg/kg (250 µM) (n=5)
- Group 4: ESWL + BH_2 2.0 mg/kg (100 µM) (n=5)

Hydrogen Peroxide (H_2O_2)

- Group 5: No-ESWL, Saline infusion (Control) (n=5)
- Group 6: ESWL, Saline infusion (Control) (n=5)
- Group 7: ESWL + BH_4 6.5 mg/kg (250 µM) (n=5)
- Group 8: ESWL + BH_2 2.0 mg/kg (100µM) (n=5)

The recorded electrical signal in pAs were converted to molar concentration using the standard curve from each calibration. For statistical analysis, all data were presented as means ±SEM. The data for each time-point in the recordings were analyzed by ANOVA using Bonferroni-Dunn. Probability values of less than 0.05 were considered to be statistically significant.

Results

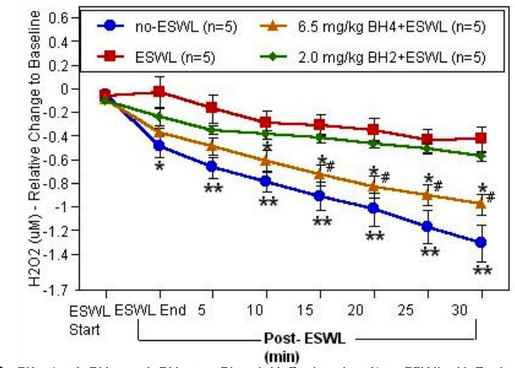

Figure 3. Effect of BH_4 and BH_2 on Blood H_2O_2 levels after ESWL. H_2O_2 levels significantly decrease post-ESWL (5-30min) for ESWL+BH_4 compared to ESWL+Saline. ESWL+BH_2 was similar to ESWL_Saline. (*p≤0.05, **p≤0.01, compared to ESWL+Saline) (#p≤0.05, ##p≤0.01, compared to ESWL+BH_2)

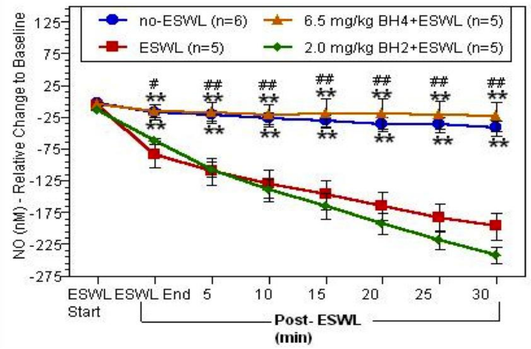

Figure 4. Effect of BH_4 and BH_2 on Blood NO levels after ESWL. ESWL+ BH_4 group is similar to non-ESWL control; significantly attenuates decreased NO levels (5-30 min post-ESWL) compared to ESWL+Saline groups. ESWL+ BH_2 group is similar to ESWL+Saline group. (*p≤0.05, **p≤0.01, compared to ESWL+Saline) (#p≤0.05, ##p≤0.01, compared to ESWL+BH_2)

Conclusions

In ESWL-treated rats, blood NO release decreases and H_2O_2 increases post-ESWL compared to non-ESWL controls. This supports our hypothesis that ESWL treatment does induce oxidative stress and NO bioavailability is reduced, therefore ESWL does cause endothelial dysfunction in the kidney. In ESWL+BH_4 treated rats, NO was significantly increased post-ESWL and H_2O_2 was significantly decreased compared to ESWL+Saline and ESWL+BH_2 group. This supports our hypothesis that infusion of BH_4 immediately post-ESWL will attenuate H_2O_2 release and increase NO bioavailability relative to ESWL+Saline. This may be due to BH_4 promoting eNOS coupling to facilitate NO release. Our ESWL+BH_2 group is similar to ESWL+Saline group. This may be due to BH_2, promoting eNOS uncoupling, which leads to increased oxidative stress with decreased production of NO. Moreover, the ESWL+BH_2 group was similar to ESWL+Saline controls regarding H_2O_2 release. This may be due to eNOS being saturated with BH_2 under ESWL conditions.

References

1. Chen, Q., Kim, E. E. J., Elio, K., Zambrano, C., Krass, S., et al. (2010). The role of tetrahydrobiopterin and dihydrobiopterin in ischemia/reperfusion injury when given at reperfusion. *Advances in Pharmacological Sciences, 2010*
2. Perkins, K. -. A., Pershad, S., Chen, Q., McGraw, S., Adams, J. S., Zambrano, C., et al. (2011). The effects of modulating eNOS activity and coupling in ischemia/reperfusion (I/R). *Naunyn-Schmiedeberg's Archives of Pharmacology,* 2011