Extracorporeal shockwave lithotripsy (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 propogates 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 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 carried 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 a 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 (H2O2) and a decrease in endothelial-derived NO bioavailability release. Under normal conditions, tetrahydrobiopterin (BH4), is the cofactor to promote eNOS coupling, and endothelial-derived NO is produced. When the tetrahydrobiopterin (BH4) to dihydrobiopterin (BH2) ratio is increased during oxidative stress, such as ESWL, BH4 promotes eNOS uncoupling and produces superoxide (SO) instead of NO. (L2) Figure 2 is then later converted to H2O2 by superoxide dismutase. BH4, and BH2, bind to eNOS with equal affinity, therefore the ratio will determine whether eNOS principally produces NO or SO.

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 H2O2 release is expected in the ESWL + Saline group compared to the non-ESWL group. When tetrahydrobiopterin (BH4) levels (mol. wt. 241.25)(Cayman Chemicals) is given at the end of ESWL treatment we predict a decrease in H2O2 release and an increase in NO release compared to ESWL + Saline group. On the contrary, when dihydrobiopterin (BH2), (mol. wt. 239.23)(Cayman Chemicals) is given at the end of ESWL treatment we predict an increase in H2O2 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 intraperitoneal injection with 2ml 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 catherization of the left renal vein with a 22-gauge catheter, the NO or H2O2 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 the decrease of one picocamp per second, indicating a stable baseline. After treatment, the stable baseline was recorded. Then, 0.5 ml of saline or drug solution was infused through the jugular vein cannulation 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.

Conclusions

In ESWL-treated rats, blood NO release decreases and H2O2 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+BH4 treated rats, NO was significantly increased post-ESWL and H2O2 was significantly decreased compared to ESWL+Saline and ESWL+BH2 group. This supports our hypothesis that infusion of BH4 immediately post-ESWL will attenuate H2O2 release and increase NO bioavailability relative to ESWL-Saline. This may be due to BH4 promoting eNOS coupling to facilitate NO release. Our ESWL+BH4 group is similar to ESWL+Saline group. This may be due to BH4, promoting eNOS uncoupling, which leads to increased oxidative stress with decreased production of NO. Moreover, the ESWL+BH2 group was similar to ESWL+Saline controls regarding H2O2 release. This may be due to eNOS being saturated with BH2 under ESWL conditions.

References