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)

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ABSTRACT

BACKGROUND: Clinical ESWL treatment to ablate kidney stones can cause acute to chronic damage in renal microvasculature leading to decreased renal blood flow and hypertension. Shockwaves can stimulate endothelial cells to release superoxide resulting in decreased nitric oxide (NO) bioavailability and increased oxidative stress, causing vascular endothelial dysfunction in the kidney. When the dihydrobiopterin:tetrahydrobiopterin ratio is increased during oxidative stress such as ESWL, eNOS becomes uncoupled and produces superoxide instead of NO. Superoxide is converted to hydrogen peroxide (H2O2) by superoxide dismutase. Protein kinase C epsilon (PKC-ε) is known to positively regulate endothelial NO synthase (eNOS) activity. In order to establish controls for the effects of PKC-ε activator and inhibitor, the effect of ESWL was tested by the comparison of ESWL-treated rats to those with no ESWL exposure, both with a saline infusion. We hypothesized that the PKC-ε peptide inhibitor (Myr-EAVSLKPT, MW = 1054.6) would decrease ESWL-induced H2O2 release and decreased the attenuation of NO release compared to ESWL-saline control rats. PKC-ε activator (Myr-NDAPIGYD, MW = 1098.5) was expected to show no effect on H2O2 or NO release, displaying a similar trend to ESWL-saline control rats.

METHODS: H2O2 or NO was measured in real-time by inserting a H2O2 or NO microsensor (100μm diameter) into the left renal vein in anesthetized Sprague-Dawley rats. ESWL treatment was administered with 16 kV shock waves for 13 minutes in a period of 500 shocks at 60 beats/min, followed by 500 shocks at 120 beats/min by a Dornier Epos Ultra HE (high-energy) lithotripter. Immediately post-ESWL treatment, saline or drug was infused through the external jugular vein.

RESULTS: ESWL-treated controls (n = 6) had shown increased H2O2 release compared to the no-ESWL controls (n = 5) and NO release in ESWL-treated rats (n = 6) was diminished compared to no-ESWL controls (n = 5) through all time points, up to 30 minutes, after ESWL treatment (H2O2, p < 0.01; NO, p < 0.001). Infusion of PKC-ε inhibitor in ESWL-treated rats significantly reduced H2O2 release (n = 6) from 15 minutes (p < 0.05) to 30 minutes (p < 0.01) after ESWL compared to ESWL-saline controls (n = 6). PKC-ε inhibitor also significantly increased NO release (n = 5) 5 minutes (p < 0.001) to 30 minutes (p < 0.001) compared to ESWL-saline controls (n = 6). Results from PKC-ε activator showed slightly increased release of both NO and H2O2, however there was no statistically significant difference to ESWL-saline controls in H2O2 release (n = 5) or in NO release (n = 5).

CONCLUSION: The data shows that inhibition of PKC-ε decreases ESWL-induced H2O2 release and increases NO release, thereby simultaneously aiding coupled eNOS and inhibiting uncoupled eNOS, ultimately resolving in increased NO bioavailability. This results in decreased oxidative stress after ESWL treatment, attenuating endothelial dysfunction and preventing further damage than necessary to the renal microvasculature.

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