Gender Differences on the Mouse Kidney Ischemia/reperfusion Injury

Kwon Moo Park*, Jee In Kim*, Youngkeun Ahn, Andrew J. Bonventre* and Joseph V. Bonventre

Renal Division, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA, Department of Anatomy, School of Medicine, Kyungpook National University, Daegu, Korea*

Background : Gender differences characterize the susceptibility or expression of many diseases. In general, the gender disparity has been interpreted primarily as reflecting estrogen-mediated protection against pathological conditions. Recent studies, however, suggest that male hormones may also play important roles in gender differences in disease susceptibility. An important endogenous modulator of I/R-induced tissue injury is nitric oxide (NO). NO, the product of NO synthases (NOSs) possesses anti-inflammatory, vasodilatory activity, and is anti-apoptotic though Akt signalling pathways. Sexual hormones are known to regulate NO synthesis. We have characterized differences in susceptibility of male and female mice to kidney I/R injury, and have studied the relationship of these differences to estrogen or testosterone.

Materials and Methods : Experiments were performed in age-matched (12-14 weeks) BALB/c (Charles River Laboratory) mice. In some studies iNOS +/+, and iNOS -/- mice (The Jackson Laboratory) were used. Kidney ischemia was induced by clamping the renal pedicle using microaneurysm clip. In some animals ovariectomy or castration was carried out 15 days before bilateral renal ischemia or sham surgery. Different groups of animals were administrated 17β-estradiol benzoate, testosterone propionate, dihydroxytestosterone, cyproterone, flutamide, tamoxifen, or vehicle by subcutaneous injection every day for 14 days prior to ischemia. In other experiments BALB/c mice were administrated L-arginine, N^{ω}-nitro-l-arginine (L-NNA) or 0.9% NaCl intraperitoneally 30 min before and after either ischemia or sham-operation. Calcium-dependent or calcium-independent NOS activity was measured using a commercial NOS activity assay kit (Cayman Chemical, USA), and values were normalized to protein amount and presented as fold increase over control. Nitrite levels in medium were measured using a commercial nitrite assay kit (Cayman Chemical, USA). Results are expressed as mean±SEM. Statistical differences among groups were calculated using analysis of variance (ANOVA). Differences ($p<0.05$) between groups were evaluated Student's t-test.

Results : Female mice are much more resistant to ischemia/reperfusion (I/R)-induced kidney injury when compared with males. Testosterone administration to females increases kidney susceptibility to ischemia. Dihydrotestosterone, which can not be aromatized to estrogen, has effects equal to those of testosterone. Castration reduces the I/R induced kidney injury. In contrast, ovariectomy does not affect kidney injury induced by ischemia in females. Testosterone reduces ischemia-induced activation of nitric oxide synthases (NOSs). Pharmacological (N^{ω}-nitro-L-arginine) or genetic (endothelial NOS or inducible NOS)

inhibition of NOSs in females enhances kidney susceptibility to ischemia. Antagonists of androgen or estrogen receptors do not affect the gender differences.

Conclusion : Although estrogen administration can partially reduce kidney injury associated with I/R, the presence of testosterone, more than the absence of estrogen, plays a critical role in gender differences in susceptibility of the kidney to ischemic injury. testosterone inhibits post-ischemic activation of NOSs through non-androgen receptor medicated mechanisms, leading to increased inflammation and increased functional injury to the kidney. These findings provide a new paradigm for design of therapies for ischemia/reperfusion injury.