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Orchiectomy reduces susceptibility to renal ischemic injury: a role for heat shock proteins

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Abstract

In previous studies we demonstrated that the presence of testosterone, rather than the absence of estrogen, plays a critical role in gender differences in kidney ischemia/reperfusion (I/R) injury. Although molecular chaperones such as heat shock proteins (HSPs) have been implicated as protective agents in the pathophysiology of I/R injury, their roles in gender differences in susceptibility to renal I/R injury remain to be defined. Here we demonstrate that orchiectomy increases the basal and post-ischemic expression of HSP-27 in kidney tubular epithelial cells, but not HSP-72, glucose-regulated protein (GRP)-78 or GRP-94 expression. Orchiectomy prevents the disruption of the actin cytoskeleton and renal functional disorders induced by I/R, when compared with intact male mice or orchiectomized mice treated with dihydrotestosterone, a non-aromatizable isoform of testosterone. Thus, the protection afforded by orchiectomy is associated with increased expression of HSP-27, a heat shock protein important for maintenance of actin cytoskeletal integrity. These findings indicate that testosterone inhibits the heat shock response and may provide a new paradigm for design of therapies for I/R injury.

Keywords: Gender; Kidney; Ischemia; HSP-27; HSP-72; GRP-78; GRP-94; Castration; Testosterone

Molecular chaperones such as heat shock proteins (HSPs) protect cells and tissues from various stresses including ischemia/reperfusion (I/R) [1–4]. I/R in kidney results in characteristic morphological changes including collapse of the actin cytoskeleton, loss of cell–cell contact, and disruption of cell integrity, resulting in loss of epithelial cell function [5–8]. Molecular chaperone proteins are associated with maintenance of cellular integrity, and inhibition of inflammatory, and immune responses, which are involved in I/R injury.

Gender differences result in differential responses to I/R injury in brain, heart, and kidney [9–12]. Recently, we reported that orchiectomy reduced kidney susceptibility to I/R and that the presence of testosterone, rather than an absence of estrogen, plays a critical role in these gender differences [13]. It has been demonstrated that sex hormones regulate HSP expression in various organs [14–16] and that HSP expression has been implicated in gender differences in susceptibility to I/R [15]. We hypothesized that lower susceptibility to I/R seen in orchiectomized mice is associated with HSPs. To test the hypothesis we determined HSP expression in the kidneys isolated from intact, orchiectomized, and dihydroxytestosterone (DHT)-treated orchiectomized mice prior to and 4 h after I/R.

In these studies, we found that orchiectomy increases the expression of HSP-27 in the tubular epithelial cells in kidneys prior to and 4 h after I/R, but has no effect on

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HSP-72, glucose-regulated protein (GRP)-78 or GRP-94. DHT treatment of orchiectomized animals resulted in prevention of this increase in HSP-27 production. Thus, the greater resistance to I/R seen in orchiectomized mice may be explained by the increased expression of HSP-27, which stabilizes the actin cytoskeleton in the proximal tubular epithelial cell, leading to reduced post-ischemic histological damages and functional impairment.

Materials and methods

Animal preparation. Experiments were performed in age-matched (12–14 weeks) BALB/c male mice. In all cases, studies were done according to approved animal experimental procedures by the Animal Care and Use Committee at Kyungpook National University and the Brigham and Women's Hospital. Each animal group consisted of more than four mice. Blood was collected from mice to determine plasma creatinine.

Animals were anesthetized with pentobarbital sodium (60 mg/kg body weight; BW, ip) prior to surgery. Thirty minutes of bilateral renal ischemia was carried out as previously described [3]. In some animals orchiectomy was carried out 15 days before bilateral renal ischemia or sham surgery as previously described [13]. Some groups of animals were administered dihydroxytestosterone (500 μg/kg BW; DHT; Sigma) or vehicle (Sesame oil; Sigma) by subcutaneous injection every day for 14 days prior to ischemia. Kidneys were harvested for Western blot analysis and immunohistochemical studies as previously described [4].

Immunoblot analysis. For the preparation of protein samples from kidney, the organ was homogenized with a Dounce homogenizer and the homogenate was prepared for Western blot analysis as previously described [3]. Immunoblot analyses were performed with anti-HSP-27, -HSP-72, -GRP-78, and -GRP-94 antibodies obtained from Upstate Biotechnology (Lake Placid, NY) and anti-ERK-1 antibody obtained from the Santa Cruz.

Renal functional parameters. Seventy microliters of blood was taken from the retrobulbar vein plexus at the times indicated on the figures. Plasma creatinine concentrations were measured using a Beckman Creatinine Analyzer II.

Immunohistochemistry. Kidneys were perfused via the left ventricle with PBS for 2 min at 37 °C and then 4% PLP (4% paraformaldehyde, 75 mM L-lysine, and 10 mM sodium periodate) fixative. Kidneys were excised, placed in PLP overnight at 4 °C until assay, and washed and stored in PBS containing 0.02% sodium azide at 4 °C. Fixed tissue was embedded in oxytetracycline compound (Sakura FineTek, Torrance, CA) and then cut into 4 μm sections using a cryomicrotome (Cryotome). Sections were mounted on microscope slides and stored at −20 °C until use. To detect HSP-27, the section was dried and washed with PBS. The section was incubated in 2% goat serum for 20 min at room temperature and treated with HSP-27 antibody in 2% goat serum overnight 4 °C. The sections were washed with PBS three times and incubated in the Texas red conjugated anti-rabbit goat IgG (Vecta Laboratories) for HSP-27 staining for 30 min at room temperature. The sections were washed with PBS, mounted with mounting solution (Vectashield, Vector Laboratories), and observed by fluorescence microscope (Nikon). For the phalloidin staining, sections were dried, washed with PBS, and incubated in Cy3-conjugated phalloidin (Sigma) for 15 min.

Statistics. Results are expressed as means ± SEM. Statistical differences among groups were calculated using analysis of variance (ANOVA). Differences between groups were evaluated Student's *t* test. Differences were considered statistically significant at a *p* value of <0.05.

Results

Orchiectomy increases basal and post-ischemic HSP-27 expression in the kidney

Since HSPs have been implicated in protection against I/R injury [1,3,4,17], we determined HSP-27 or HSP-72 expression in intact, orchiectomized, and DHT-treated orchiectomized mice prior to and 4 h after ischemia. The level of HSP-27 expression in orchiectomized males is greater than in intact male mice prior to ischemia (Fig. 1C). DHT administration to orchiectomized mice prevents the increase in HSP-27 expression prior to ischemia (Fig. 1C). Ischemia increases HSP-27 expression in all experimental animal groups at 4 h after ischemia (Figs. 1A and C). The post-ischemic increase of HSP-27 expression is significantly greater in orchiectomized mice than in intact males (Figs. 1B and C). DHT administration to orchiectomized mice attenuates the post-ischemic increase of HSP-27 expression (Fig. 1C).

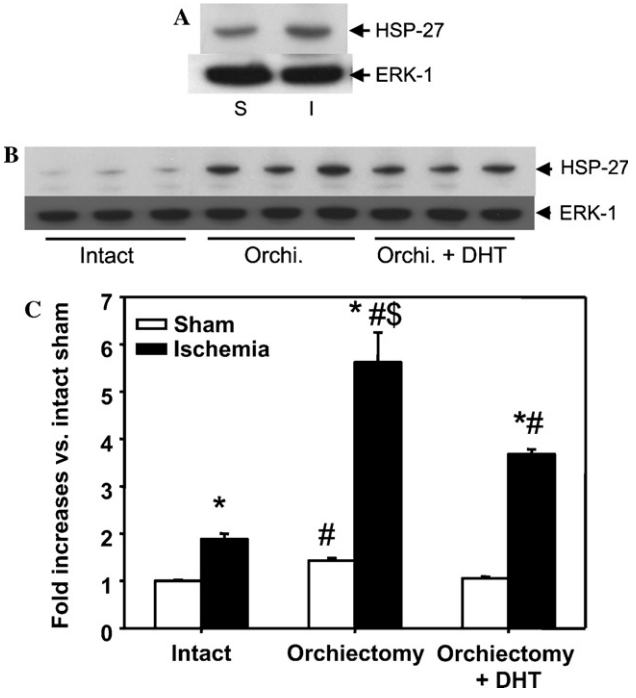

Fig. 1. Effect of hormonal modification on HSP-27 expression in the kidney. Some BALB/c mice were orchiectomized (Orchi.) on day 0. Some mice were administered dihydrotestosterone (DHT, 500 μg/kg BW) or vehicle by subcutaneous injection daily for 14 days. On day 15 mice were subjected to 30 min of bilateral ischemia at 36–38 °C. (A) The expression of HSP-27 in intact males was measured 4 h after either sham-operation (S) or 30 min of bilateral renal ischemia (I) by Western blot analysis using HSP-27 antibody. (B) Post-ischemic expression of HSP-27 in the kidney at 4 h after ischemia. ERK-1 was used as an equal loading marker. (C) The density of Western blot bands was quantified by the NIH Image program. Values presented are expressed as means ± SEM ($n = 4$). $^{*}p < 0.05$ versus their respective control. $^{\#}p < 0.05$ versus intact males before ischemia. $^{\$}p < 0.05$ versus post-ischemic expression in DHT-treated orchiectomized males.