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Exercise preconditioning reduces acute ischemic renal injury in Hsp70.1 knockout mouse

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Summary. Backgroun/aims: Heat shock protein 70 (Hsp70) is an anti-apoptotic protein that has a protective effect in renal ischemic injury. Exercise up-regulates Hsp family proteins (Hsps), antioxidants and anti-apoptotic proteins. We hypothesized that exercise as preconditioning could attenuate acute renal dysfunction and apoptosis resulting from renal ischemic injury under the Hsp70 deficient circumstance and could contribute to the prevention of renal injury induced by ischemia.

Method: To investigate the effect of exercise preconditioning on protecting the kidney from ischemia in Hsp70 deficiency, we measured apoptosis-related factors and Hsps. Hsp70.1 KO and wild-type mice were divided into sham control (Sham), exercise preconditioning (Ex), renal ischemia-after-exercise (Ex+IR), and ischemia (IR) groups. Where appropriate a treadmill exercise was performed at 20 m/min, 60 min per day on a 0% gradient for 7 days, and renal ischemia was induced by clamping the renal pedicle for 25 min. To characterize the effects of exercise on oxidative stress- and apoptosis-related factors, and Hsp27 and 70 expressions, we performed immunohistochemistry, western blotting and TUNEL assay, and also measured level of serum creatinine.

Results: Serum creatinine concentration and 4-HNE expression were raised in the IR group, as were caspases 3, 7 and 9, while Cu- and Mn-SOD levels were reduced, as were those of anti-apoptotic proteins Bcl-XL, Bcl-2 and Hsp27. All these effects were largely reversed by the exercise preconditioning, as was the decrease in apoptotic cells observed after exercise preconditioning.

Conclusion: Exercise preconditioning has a beneficial effect in inhibiting oxidative stress and apoptotic cell death in the kidney resulting from ischemic injury even under Hsp70 deficiency.

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Introduction

Vascular damage resulting from renal ischemia-reperfusion injury produces oxygen free radicals and reactive oxygen species (ROS) which induce apoptotic cell death and renal damage, and increase the morbidity and mortality of renal diseases such as acute renal failure (ARF) (Fujimura et al., 1998; Jones and Lee, 2007). Oxygen free radicals lead to protein and DNA damage by initiating lipid peroxidation mediated by toxic breakdown products such as malondialdehyde and 4-hydroxynonenal (4-HNE) (Ueda et al., 1996). However, some enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase protect cells from oxidative damage. Two types of SODs, Cu/Zn-SOD and Mn-SOD, metabolize superoxide radicals, so inhibiting the production of hydroxyl groups, and reduce the conversion of superoxide anions into hydrogen peroxide and oxygen (Adams and Best, 2002). Hence, SODs are considered easily analyzable indicators of responses to oxidative stress. Since anti-apoptotic factors such as Bcl-2 and -XL inhibit the cellular damage provoked by activation of apoptotic caspases, stimulating the levels of SOD and anti-apoptotic proteins can in principle be useful in preventing ARF. However, no widely effective treatment of ARF has yet been developed (Jones and Lee, 2007).

Recently, the induction of heat shock proteins (Hsps), especially Hsp70 including 27, has been reported to protect against renal injury from ischemia and its cross-linked renal function and apoptosis (Wang et al., 2011). Hsps are inducible proteins reactive to several stresses including oxidative stress (Chien et al., 2001; Pokly, 2001). They are subdivided into Hsp27, 60, 70, 90 and 110 according to molecular weight. Hsp70

functions as a chaperon promoting the folding of unstable proteins in the cytoplasm (Georgopoulos and Welch, 1993), and increased level of Hsp70 can protect cells from fatal extrinsic damage. Hsp27 is also a potent anti-apoptotic protein with similar cytoprotective effects to Hsp70 (Wang et al., 2011) and is a key modulator of oxidative stress and regulator of cytoskeleton (Concannon et al., 2003).

Exercise is one method of up-regulating the expression of Hsp70 (Locke and Noble, 1995), and has a similar effect to ischemic preconditioning in protecting against tissue damage from ischemia (Hartmann and Bung, 1999; Ding et al., 2006). Exercise induces Hsps expression, which opposes weight gain and hypoxia, increases antioxidant enzymes, and inhibits DNA damage and cellular apoptosis from oxidative stress (Fahrenbach and Northoff, 2001; Pokly, 2001). In particular, Hsp70 over-expression protects against tissue damage induced by ischemia (Marber et al., 1995; Rajdev et al., 2000), and increased Hsp70 expression resulting from aerobic exercise inhibits the apoptosis of cardiomyocytes (Moran et al., 2004). Therefore, exercise may contribute to anti-apoptotic protein production against renal ischemic injury. However, direct and indirect effects of exercise on renal ischemic injury remain unknown under Hsp70 deficient state.

The aim of this study is to investigate the effect of exercise as preconditioning in preventing renal injury induced by ischemia, and which mechanisms are involved in the protection of the kidney from ischemic injury under an Hsp70 deficient environment. In order to do this, we used Hsp70.1 knockout (KO) and wild-type (WT) mice as experimental animals, and analyzed blood samples, performed western blotting to assess levels of SODs and apoptotic and anti-apoptotic factors, and immunohistochemistry of 4-HNE as an oxidative stress marker, and carried out TUNEL assay.

Materials and methods

Animals and exercise protocol

Eight-week-old male Hsp70.1 KO and WT mice were used; they were purchased from Macrogen Laboratory (Seoul, Korea) as described previously (Lee et al., 2001). The animal care and experimental procedures were approved by the Animal Care Committee of Hanyang University. Mice were kept at constant temperature and humidity with a 12:12h dark-light cycle, housed at 21°C, and given free access to food and water throughout the study period. After one week period of acclimation, the mice were divided into the following groups: sham control (Sham, n=8), exercise (Ex, n=8), ischemia-after-exercise (Ex+IR, n=8), and ischemic (IR, n=8) group.

Pre-exercise was conducted for 5-30 min/day over 5 days so that the mice became familiar with the treadmill exercise environment. After this period, treadmill exercise training was given for 20 m/min, 60 min per day on a 0% gradient for 7 consecutive days, as described previously (Akita et al., 2007).

Surgery and experimental protocol

Mice were anesthetized with ketamine-lumpun (50 and 40 mg/kg, respectively, diluted in saline) by the intraperitoneal route and placed on a heating pad at 38°C to maintain constant body temperature. To induce ischemia, flank incision was performed and the kidneys were exposed. The renal pedicle including renal artery and vein was clamped for 25 min, and the kidney was covered with saline gauze during the ischemia. Thereafter the clamp was removed, restoration of blood flow was checked, and warm saline was instilled into the flank. The sham group was subjected to sham operation without ischemia. Flank was closed with muscle wall and skin, and all the mice were killed 1 hour after renal ischemia-reperfusion injury or sham surgery.

Blood sampling and measurement of serum creatinine

Blood was taken by heart puncture to measure creatinine level. Serum was isolated by centrifuging the blood samples, and creatinine level was measured with a creatinine assay kit (Vitros, Johnson & Johnson, Langhorne, PA, USA).

Histopathological studies

The kidney tissues were fixed in 4% para-formaldehyde and 0.05% glutaraldehyde in phosphate buffered saline (PBS) for 2 h at 4°C, washed, dehydrated in ethanol, and embedded in paraffin. Then, 6 μm thick sections were cut, and all tissue sections were deparaffinized, cleared, and hydrated to PBS using a descending series of ethanol concentrations. Some sections were stained with hematoxylin and eosin for routine histopathological analysis. The brush border in the proximal tubular cells was observed with periodic acid-Schiff (PAS) reaction.

4-HNE Immunohistochemistry

Tissue sections were deparaffinzed, cleared, and hydrated to PBS using a descending series of ethanol. The sections were blocked for 40 min at 37°C with 3% goat serum in PBS followed by quenching endogenous peroxidase activity by exposing slides to 0.3% H_2O_2 and 10% methanol for 5 min. Primary antibodies for 4-HNE (1:250) (abcam, Cambridge, UK) were added and incubated overnight at 4°C. The next day, the slides were washed in PBS three times for 5 min and secondary antibody (1:200, Santa Cruz Biotechnology, CA, USA) was incubated for 1 h at room temperature, and then washed in PBS several times for 3 min before DAB visualization. Finally, the slides were counterstained with 0.5% methyl green and observed on a light microscope (Olympus U-LH 100HG, Tokyo, Japan). For

negative controls the primary antibodies were omitted.

Western blotting

Renal cortex was homogenized in lysis buffer (Cell Signaling Technology, Danvers, MA, USA) with PMSF at 4°C and centrifuged (13,000xg). The protein content of each sample was determined by the Bradford method with bovine serum albumin as a standard. Protein samples (35 μg) were boiled with 5x sample buffer, electrophoresed on polyacrylamide gels, and transferred to nitrocellulose membranes at 15V over night. The membranes were washed, blocked, and incubated with antibodies to detect Cu-SOD, Mn-SOD (1:1500; Chemicon, Temecula, CA, USA), cleaved caspase-9, -7ß-3 (1:1000; cat. #9664, #9491, #9509, Cell Signaling Technology, Danvers, MA, USA), Bcl-2, Bcl-XL (1:1000; Cell Signaling Technology, Danvers, MA, USA), and Hsp27 and 70 (1:1000; Stressgen, Ann Arbor, MI, USA) for 12 hr at 4°C. HRP-conjugated secondary antibody (1:5000; Santa Cruz Biotechonology, Santa Cruz, CA, USA) was added for 1 hr at room temperature. The membranes were washed and visualized by autoradiography after development with an ECL Plus Kit (GE Healthcare Bio-Sciences Crop, Piscataway, NJ, USA). ß-actin was used as internal control. Densitometry was performed with gel documentation equipment (Gel Doc 2000, Quantity One, Bio-Rad, Hercules, CA, USA).

Detection of apoptosis by TUNEL assay

The kidney tissues were fixed by immersion in 4% paraformaldehyde 0.05% glutaldehyde in PBS. After fixation, the samples were dehydrated in ethanol and embedded in paraffin. Transverse sections of 6 μm were cut and deparaffinized in xylene, and TUNEL staining was performed with an Apo Tag Peroxidase In Situ Apoptosis Detection Kit (Chemicon, Temecula, CA, USA) according to the manufacturer's protocol. Briefly, the slides were incubated with proteinase K in PBS for 15 min followed by two washes in distilled water (DW). They were then quenched in 0.3% H_2O_2 in equilibration buffer for 10 min, and incubated in TdT enzyme for 1 hr at 37°C, after which they were put in stop/wash buffer for 10 min. Next, the slides were rinsed in PBS three times and anti-digoxigenin peroxidase conjugate was applied to the tissues for 30 min at room temperature. After washing in PBS three times, peroxidase activity was visualized using a DAB substrate kit (Vector Laboratories, Burlingame, CA, USA). For negative controls, the TdT enzyme was omitted. Finally, the slides were observed with a light microscope (Olympus U-LH 100HG, Tokyo, Japan).

Scoring method of the immunohistochemistry and apoptosis

The immunoreaction of 4-HNE and numbers of apoptotic cells were analyzed with an image analyzer (Analysis Pro 3.2, Sis Co., Munster, Germany). Seven slides were randomly selected from the kidney section.

Statistical analysis

All data were expressed as means ± SEM and analyzed by two-way ANOVA (GraphPad software, GraphPad, La Jolla, CA, USA) using the procedures in the SPSS software (SPSS Inc, 12.0, Chicago, IL, USA), with Bonferroni post tests. $p<0.05$ was considered statistically significant.

Results

Measurement of serum creatinine

To see whether acute renal ischemia causes renal dysfunction and serum creatinine is raised, Hsp70.1 KO and WT mice were subjected to bilateral renal pedicle clamping for 25 min. As shown in Fig. 1, serum creatinine level of Hsp70.1 KO increased to 0.61±0.11 mg/dl in the ischemic group (IR) compared with 0.2±0.09 mg/dl in the Sham group, and Ex+IR group almost completely prevented the rise in creatinine (0.27±0.08 mg/dl). In WT mice, creatinine level of IR group (0.21±0.05 mg/dl) was lower than IR group of Hsp70.1 KO, and exercise preconditioning also decreased creatinine production.

Histopathological analysis

Histopathlogical analysis was performed with routine hematoxylin and eosin stain (Fig. 2A), and periodic acid-Schiff (PAS) reaction (Fig. 2B) was used for observation of brush border in the proximal tubular cells. The kidneys of IR group showed serious tubular

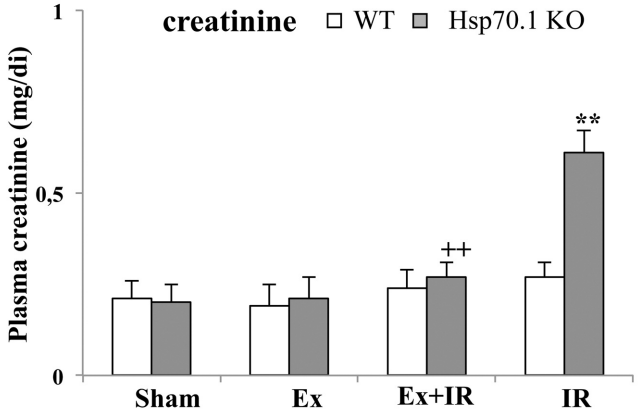

Fig. 1. Serum creatinine concentrations. ** ($p<.01$) denotes a significantly higher creatinine concentration in the IR group than in the Sham group. ++ ($p<.01$) denotes a significantly lower creatinine concentration in the Ex+IR group than in the IR group. Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemic injury.

damage. Dilatation of proximal tubule, obstruction of tubular space with cellular debris, widened capsular space and extensive loss of brush border were found in IR group of both WT and KO mice. However, kidneys of Ex+IR group displayed nearly normal tubular morphology with intact or less loss of brush border and cellular debris, and well-preserved tubular structure.

Effect of exercise preconditioning on expression of 4-HNE

4-HNE as an oxidative stress marker was examined by immunohistochemistry and scored. As shown in Fig. 3, 4-HNE was highly expressed in the proximal tubular cells. 4-HNE expression in the Hsp70.1 KO mice was significantly higher in IR group; however, Ex+IR group showed markedly lower expression than IR group. Although the level of 4-HNE expression in WT mice was generally lower than Hsp70.1 KO, the overall expression pattern was similar between both groups.

Effect of exercise preconditioning on expression of SOD proteins

Mn-SOD and Cu-SOD levels were examined by western blotting. As shown in Fig. 4, Cu-SOD and Mn-SOD expression in the Hsp70.1 KO and WT mice was down-regulated after ischemia, and exercise largely prevented this down-regulation.

Effects of exercise preconditioning on expression of heat shock proteins

Hsp27 and 70 were examined by western blotting.

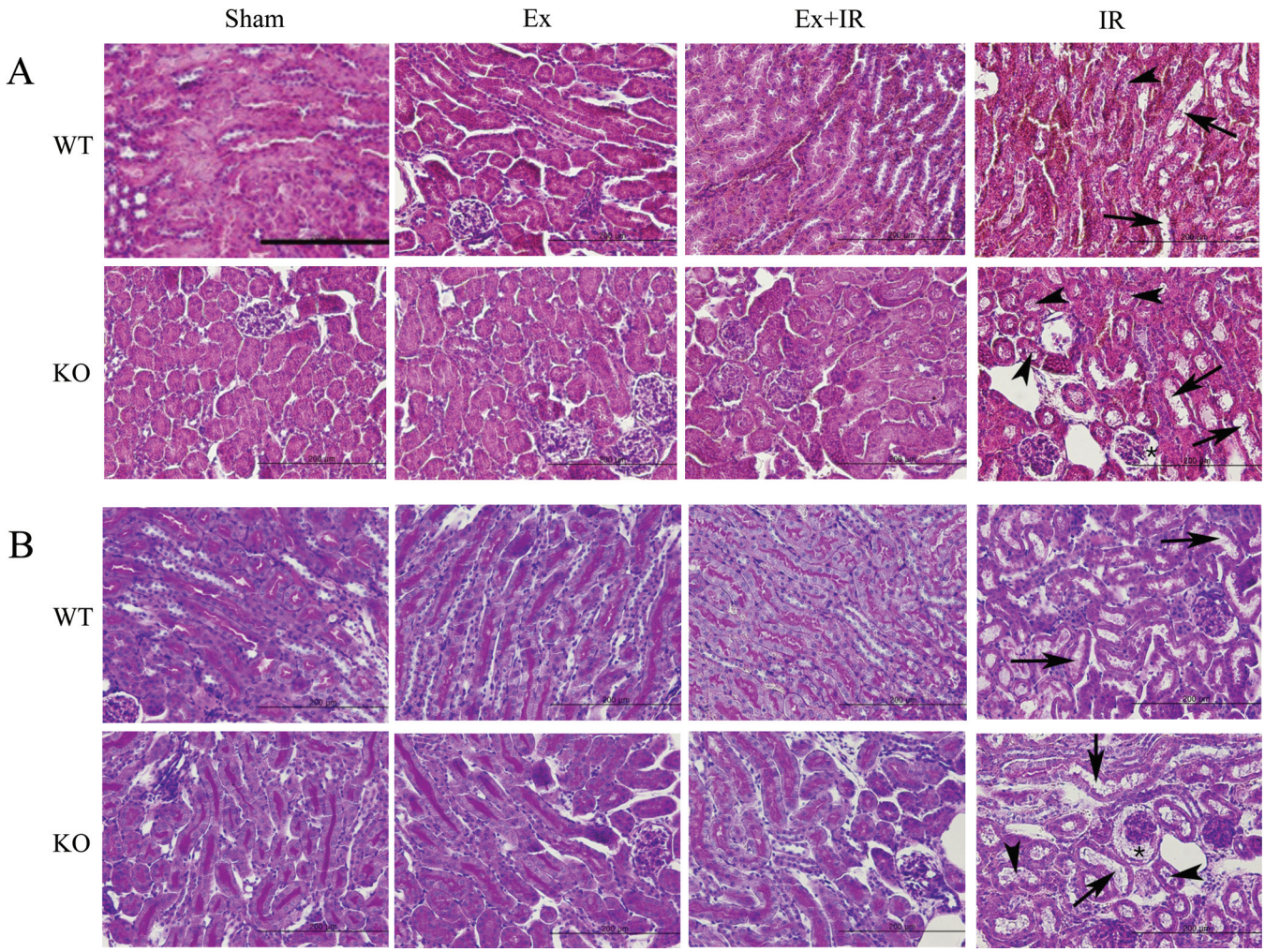

Fig. 2. Representative histopathological findings of the renal cortex. Hematoxylin and eosin staining **(A)** and PAS reaction **(B)**. Distended proximal tubule (arrow), cellular debris in tubular lumen (arrow head), widened capsular space (asterisk) and extensive loss of brush border were found in the IR group of both WT and KO mice; however, most of the proximal tubule of Ex+IR group were similar to the control group. Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemia group. Scale Bars. 200 μm.

As shown in Fig. 5, Hsp27 and 70 expressions of Hsp70.1 KO were lower than WT mice. Hsp27 expression of Hsp70.1 KO was reduced in the IR group but recovered in the Ex groups, while Hsp70 expression of Hsp70.1 KO was virtually undetectable in all groups. However, in WT mice, Hsp70 expression of IR group was up-regulated compared to Sham, and exercise preconditioning significantly increased Hsp70 expression. In addition, although the intensity of Hsp27 expression in Hsp70.1 KO mice was lower than that of WT mice, general patterns of expression between groups were similar between both animals.

Effects of exercise preconditioning on expression of apoptosis-related proteins

Expression of the anti-apoptotic proteins, Bcl-2 and Bcl-XL, was significantly reduced in the IR group, and this effect was largely prevented by exercise preconditioning (Fig. 6). Similarly, expression of caspase-9, -7 and -3 proteins increased significantly in the IR group and exercise preconditioning greatly reduced this increase, especially in the cases for caspase-9 and -3 (Fig. 7).

Effects of exercise preconditioning on apoptotic cell death

To examine morphologically the effect of exercise on apoptotic cell death after ischemic exposure, we used TUNEL assays. Many apoptosomes were observed in proximal tubular cells near corticomedullary junction in the IR group, and their numbers were considerably diminished in the Ex+IR group. However, Hsp70.1 KO mice showed more apoptotic cells in the renal tubule than WT mice (Fig. 8).

Discussion

The mechanisms of renal injury induced by ischemia are diverse and complex; however, some important factors have been suggested recently. Increasing Hsp70 either before or after ischemic injury preserves renal function by attenuating acute kidney injury (Wang et al.,

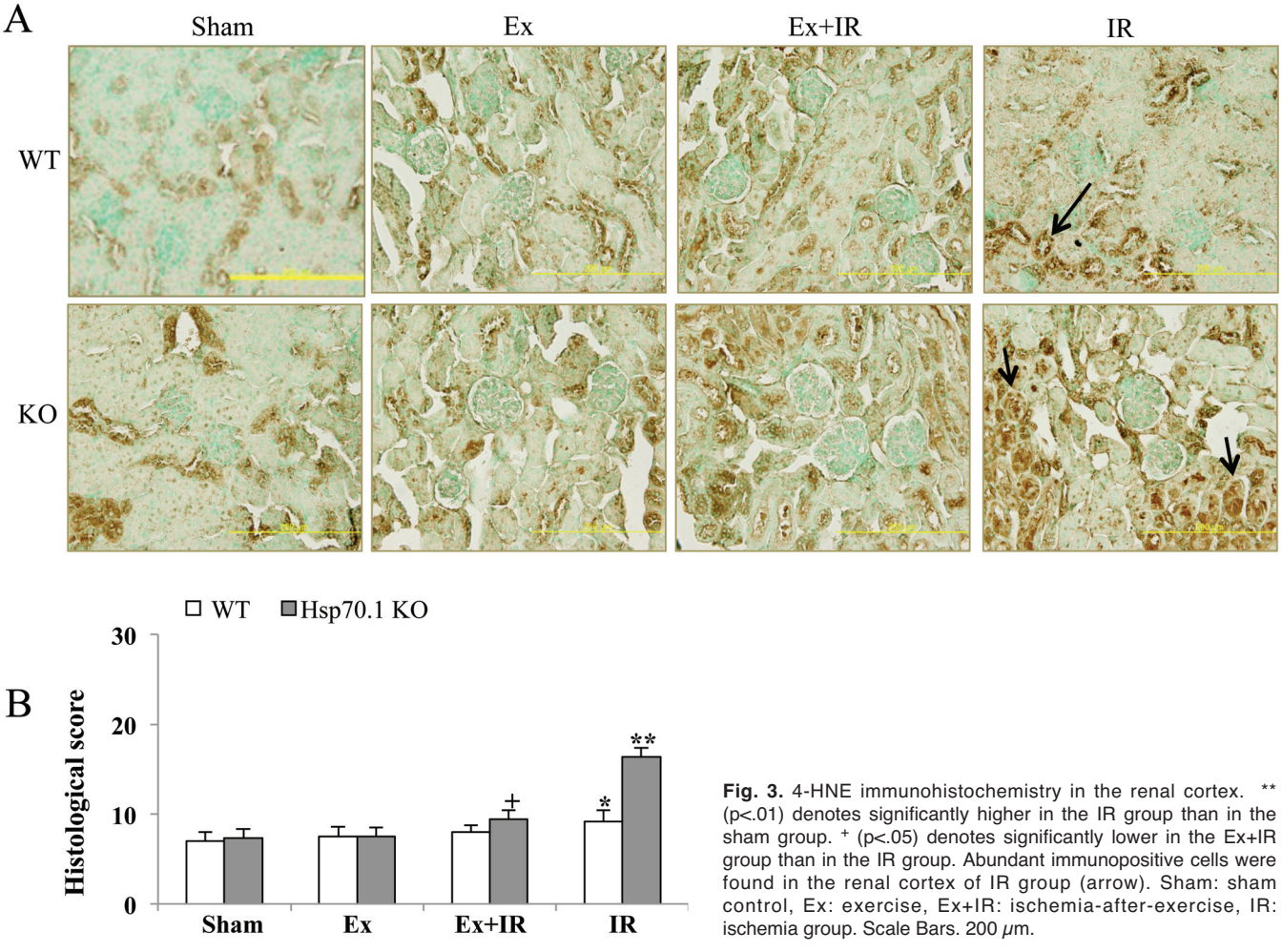

Fig. 3. 4-HNE immunohistochemistry in the renal cortex. ** ($p<.01$) denotes significantly higher in the IR group than in the sham group. $^{+}$ ($p<.05$) denotes significantly lower in the Ex+IR group than in the IR group. Abundant immunopositive cells were found in the renal cortex of IR group (arrow). Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemia group. Scale Bars. 200 μm.

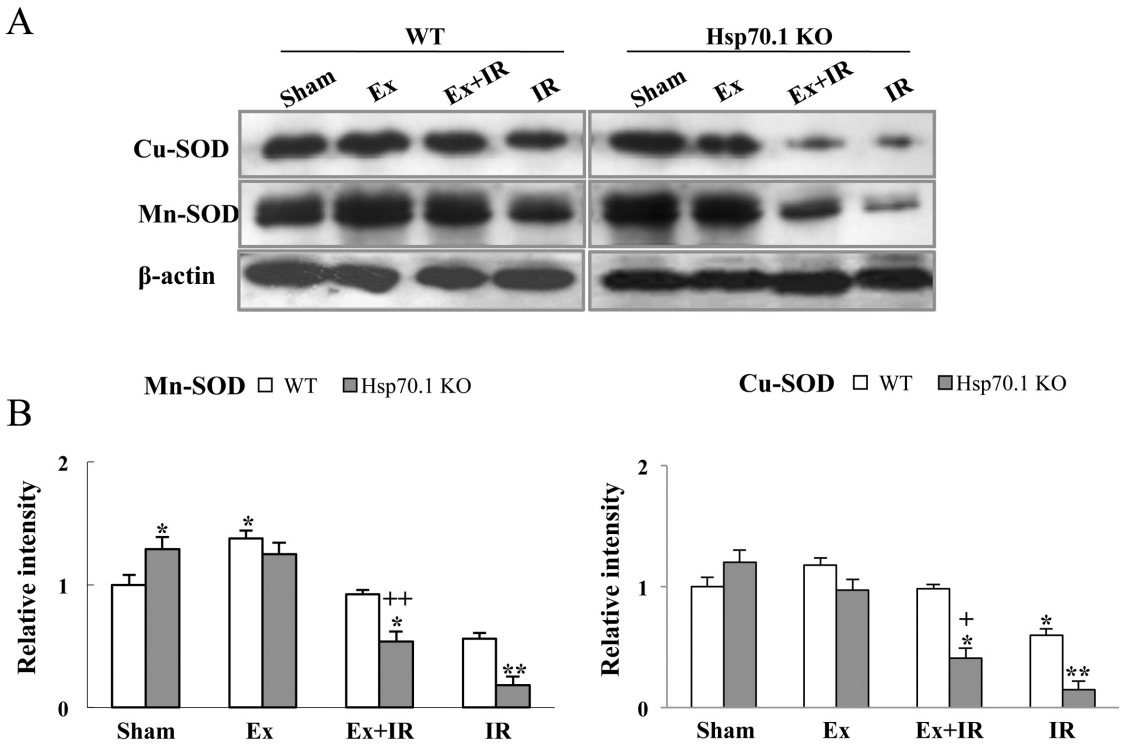

Fig. 4. Expression of Cu-SOD and Mn-SOD proteins **(A-C)** in the kidney. ** ($p<.01$) denotes significantly lower Cu-SOD and Mn-SOD in the IR group than in the sham group. $^{+}$ ($p<.05$), $^{++}$ ($p<.001$) denote significantly higher Cu-SOD and Mn-SOD in the Ex+IR group than in theIR group. Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemic injury.

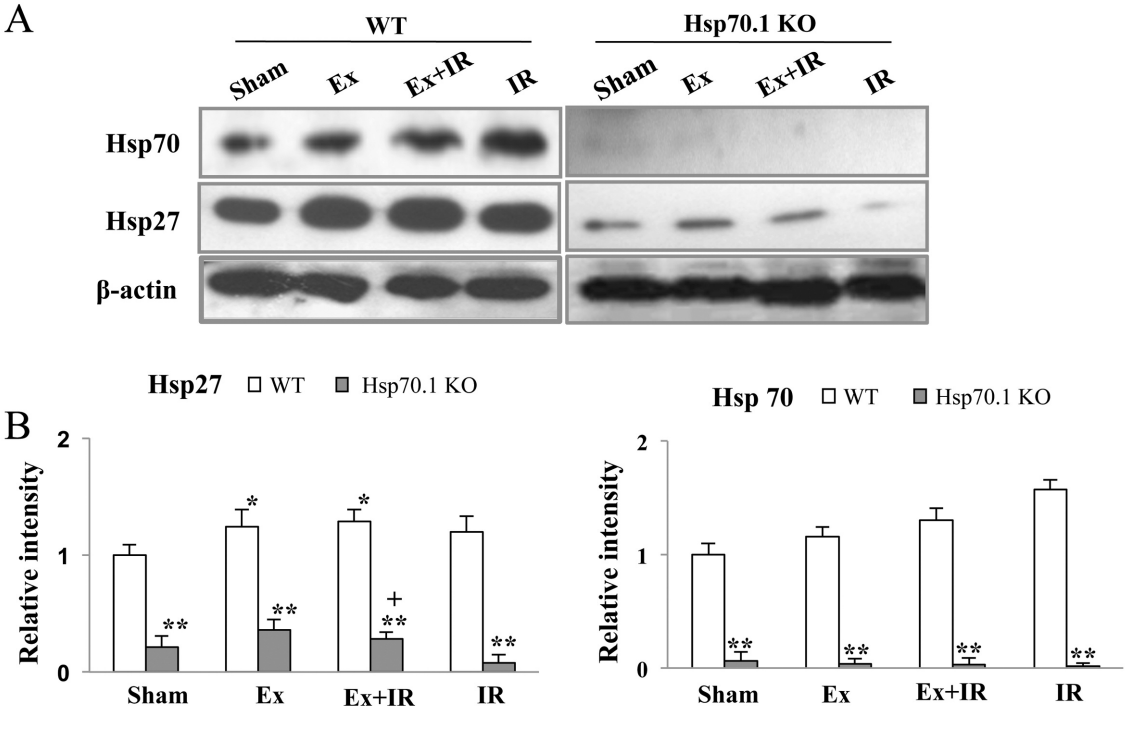

Fig. 5. Expression of Hsp27 and 70 proteins in the kidney. * ($p<.05$) denotes significantly lower Hsp27 than in the sham group. $^{+}$ ($p<.05$) denotes significantly higher Hsp27 in the Ex+IR group than in the IR group. Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemic injury.

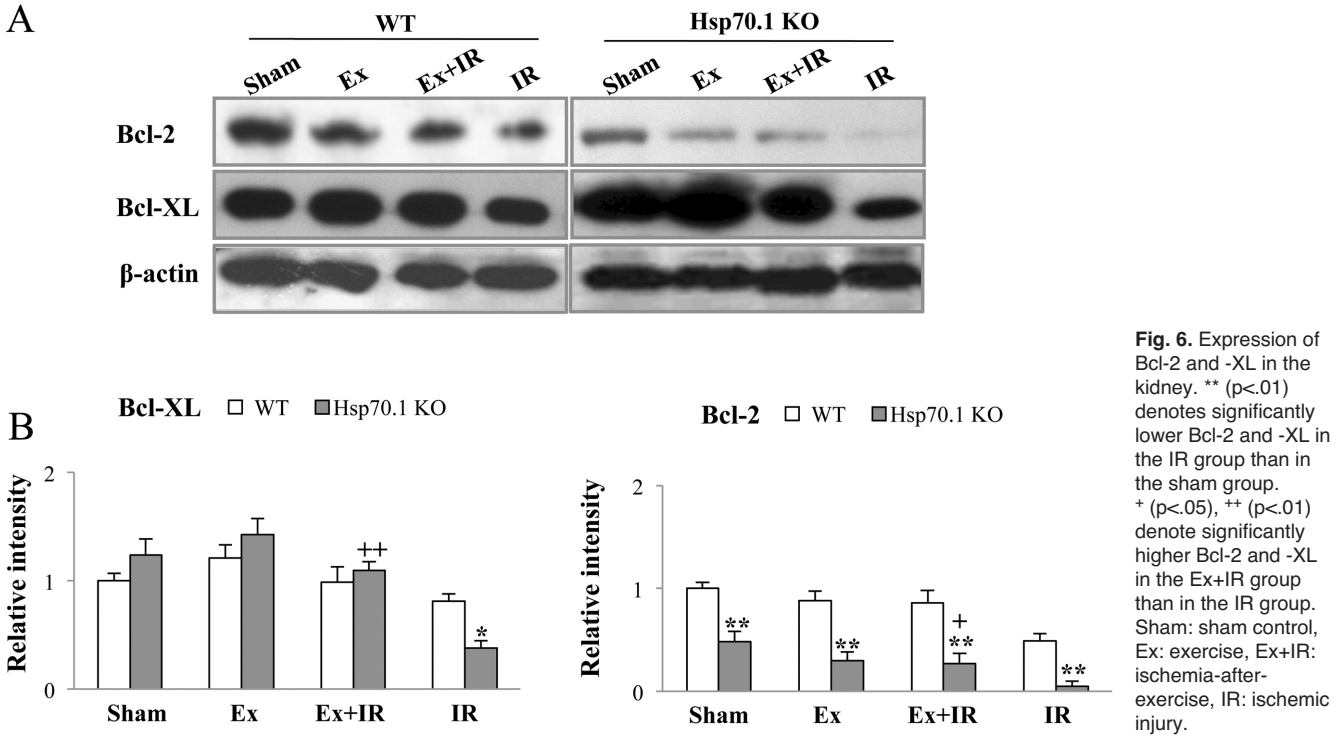

Fig. 6. Expression of Bcl-2 and -XL in the kidney. ** (p<.01) denotes significantly lower Bcl-2 and -XL in the IR group than in the sham group. + (p<.05), ++ (p<.01) denote significantly higher Bcl-2 and -XL in the Ex+IR group than in the IR group. Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemic injury.

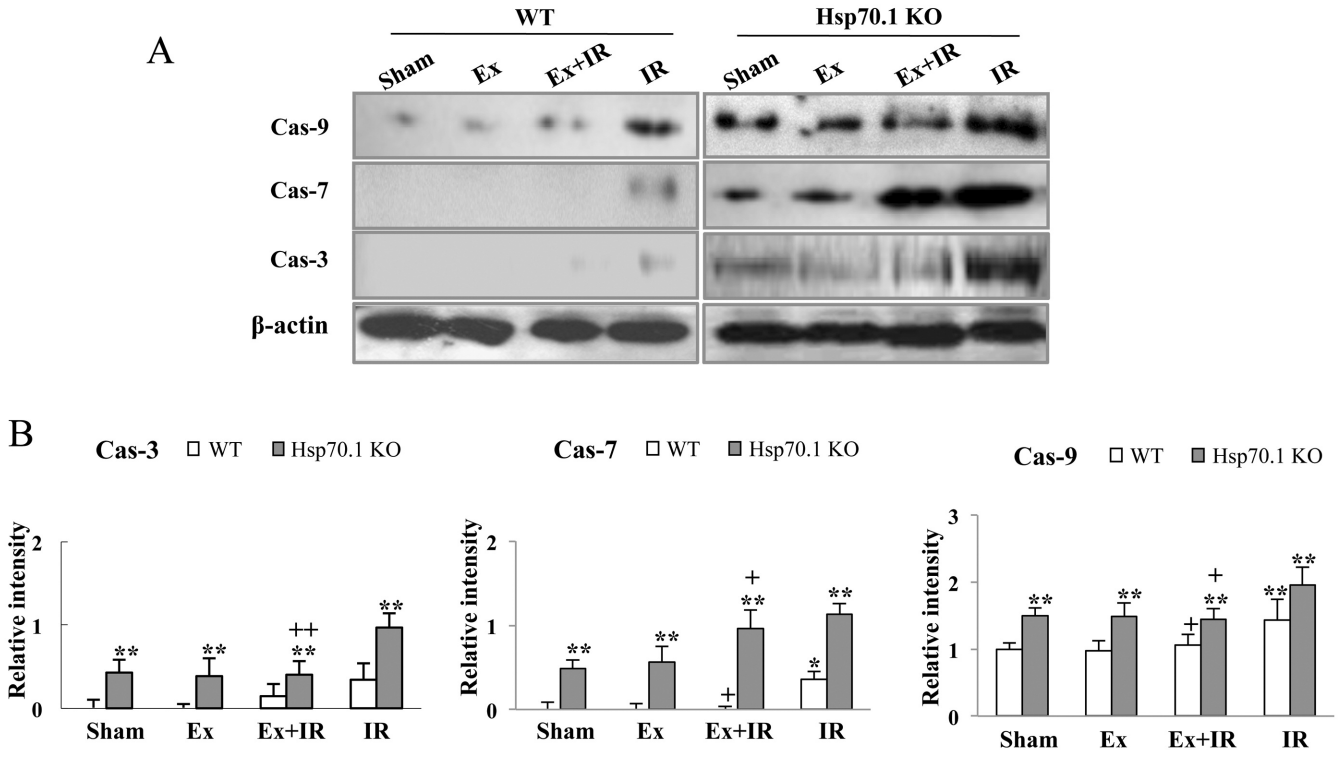

Fig. 7. Expression of caspase-9, -7 and -3 proteins in the kidney. * (p<.05), ** (p<.01) denote significantly higher caspase-9, -7 and -3 in the IR group than in the sham group. + (p<.05), ++ (p<.01) denote significantly lower caspase-9, -7 and -3 in Ex+IR group than in the IR group. Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemic injury.

2011). Hsp70 stabilizes protein structures and microfibers to protect the kidney from ischemia-reperfusion (Huot et al., 1996); it also reduces oxidative stress by decreasing lipid membrane degradation resulting from the ischemia (Beck et al., 2000; Snoeckx et al., 2001). Hsp70 is induced by stimuli such as hypoxia, cellular damage, hyperthermia and oxidative stress (Kregel, 2002). It functions as a chaperone to inhibit apoptosis (Christians et al., 2003) by blocking release of Bax from mitochondria, inhibiting the formation of apoptosome complex, activation of Bid, and migration of AIF from mitochondria to the nucleus (Barrier et al., 2009). Hsp70 abundant experimental rat (Brown Norway rat) is resistant to acute ischemic kidney injury (Basile et al., 2004). In addition, Hsp70 targets mitochondria-mediated apoptosis (Jayakumar et al., 2001). Thus, inhibition of Hsp70 expression results in apoptosis, accelerating ischemic renal injury (Wang et al., 2011). Meanwhile, the mice have two genes of Hsp70 (*hsp70.1 and hsp70.3*) which show 99% homology; however, both genes are considerably different in the 3' untranslated region, although they produce Hsp70 protein (Lee et al., 2004). A reduced Hsp70 protein level in *hsp70.1* KO mice increases cellular damage during acute focal cerebral ischemia (Lee et al., 2001). The deletion of the *hsp70.1* gene increases cytochrome c release into the cytoplasm and subsequent caspases-3 activation, thereby exacerbating apoptosis after focal cerebral ischemia (Lee et al., 2004). In addition, antioxidant enzyme Cu/Zn SOD protein level in *hsp70.1* KO mice is lower than that of WT (Choi et al., 2005). Thus, *hsp70.1* KO mice are more vulnerable to ischemic injury and have been used as experimental animals in the present study.

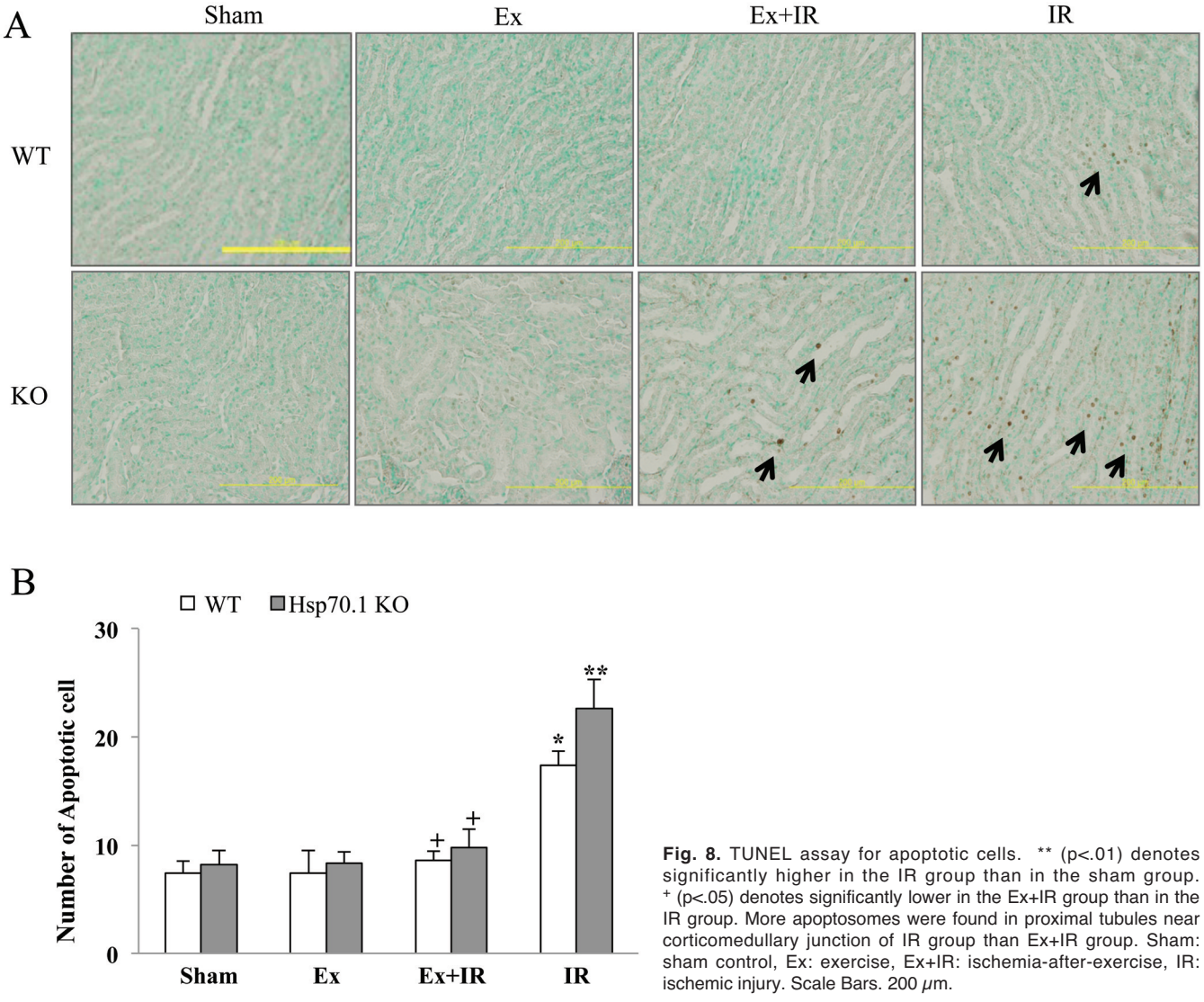

Fig. 8. TUNEL assay for apoptotic cells. ** (p<.01) denotes significantly higher in the IR group than in the sham group. + (p<.05) denotes significantly lower in the Ex+IR group than in the IR group. More apoptosomes were found in proximal tubules near corticomedullary junction of IR group than Ex+IR group. Sham: sham control, Ex: exercise, Ex+IR: ischemia-after-exercise, IR: ischemic injury. Scale Bars. 200 μm.

Exercise improves tolerance to ischemia and attenuates the tissue damage caused by ischemia-reperfusion regardless of the exercise period (Hartmann and Bung, 1999; Ding et al., 2006). Swimming exercise during pregnancy improves placental function and enhances Hsps expressions (Powers et al., 1998). Exercise also protects against cell death from DNA damage by up-regulation of Hsp70 and Hsp27 through p38 signaling pathway (Fehrenbach and Northoff, 2001; van Ginneken et al., 2006). Moreover, exercise reduces hyperthermia-induced apoptosis in developing mouse brain (Lee et al., 2011). Until recently, however, the mechanisms by which exercise defends against renal tissue injury have not been fully understood, especially in the absence of Hsp70.

Ischemia induces ROS production, which is accentuated in Hsp70 deficiency. Because Hsp70 deficiency inhibits the expression of the antioxidant enzyme SOD (Choi et al., 2005), the production of ROS causes lipid peroxidation, DNA damage and protein dysfunction, resulting in structural and functional damage to the kidney (Seok et al., 2007; Chen et al., 2009). Ischemia also exhausts ATP reserves in kidney cells, impairing cytoskeletal and membrane-attached proteins, and activates ion pumps in the plasma membrane and cytoplasmic organelle membranes, activating phospholipases and proteinases and causing kidney damage (Brady et al., 2004). However, exercise increases levels of the mitochondrial enzymes regulating oxidative metabolism in the mouse (Nakao et al., 2000), and increases antioxidant defenses against the ROS produced by stress (Ji, 2002). In addition, exercise has been reported to induce vascular dilation by increasing nitric oxide levels in vascular endothelium and to reduce DNA damage by increasing levels of antioxidant enzymes and Hsp27 and 70 in heart and skeletal muscle (Arakawa, 1993; Fahrenbach and Northoff, 2001; Husain and Bazelrigg, 2002; van Ginneken et al., 2006; Lunz et al., 2011). In the present study we showed that ischemia-reperfusion for 25 min reduced antioxidant enzymes Cu- and Mn-SOD expressions, and greatly increased oxidative stress marker 4-HNE in Hsp70.1 deficient mice, and these effects were opposed by exercise preconditioning.

Stress-induced apoptosis is largely dependent on a balance between apoptotic Bax and anti-apoptotic Bcl-2 and -XL. If this balance is broken by stress, cytochrome C is released from mitochondria and activates the caspase cascade, inducing apoptosis. Moreover, ROS acts downstream of cytochrome C and upstream of caspase-3 and causes apoptotic cell death (Li et al., 2000). The anti-apoptotic proteins, Bcl-2 and -XL, regulate apoptotic signaling by preventing cytochrome C release and inhibiting activation of downstream caspases activation (Salvensen and Dixit, 1997). These proteins also play a central role in the delivery of apoptotic signals to the mitochondria in stress-induced apoptosis (Delchev et al., 2006). To identify how exercise preconditions cells against ischemia, we studied the expression of apoptosis-related proteins, Hsp27 and 70 by western blotting. Under conditions of Hsp70.1 deficiency, expression of caspase-9, -7 and -3 was increased, and that of Bcl-2, -XL and Hsp27 decreased by ischemia, and these effects were opposed by exercise. Our results suggest that ischemia increases ROS production, which causes oxidative damage to mitochondrial membranes and impairs cell function by activating caspase-9 through caspase-3 (Pan et al., 2008). Decreases of Bcl-2 and -XL after renal ischemia are associated with caspase-3 activation in Hsp70.1 deficiency, because Hsp70 regulates members of the Bcl family and inhibits the activation of caspase 3 (Wang et al., 2011). However, in the present study, exercise preconditioning up-regulated Hsp27 expression after ischemic injury. Hsp27 is an ATP-independent chaperone, functioning especially in protection against protein aggregation (Ehmsperger et al., 1997). Exposure of cells to high temperature (43°C for 3 h) stimulates expression of Hsp25/27 and induces a thermoresistant state (Geum et al., 2002). Likewise, our laboratory has demonstrated that regular exercise protects the developing brain against hyperthermia by up-regulating Hsp27 expression in Hsp70.1 KO mice (Lee et al., 2011). In addition, Hsp27 can inhibit apoptosis by direct inhibition of caspase activation (Concannon et al., 2003). Thus, exercise preconditioning prevents caspase activation by increasing Hsp27, Bcl-2 and -XL, and protects cells from the effects of ischemia. Meanwhile, in the present study, Hsp27 expressions in Hsp70.1 KO mice were lower than those in WT mice. Although we do not know the exact mechanism of this result, there are recent studies which may support our finding. Sreedharan et al. (2011) have studied the cytoprotective function of Hsp27 in an *in vitro* experiment and suggested that Hsp27 is dependent on Hsp70 to provide its maximal cytoprotective effect, and Hsp25/27 must have coordinated activity with other Hsp classes, especially Hsp70. In addition, our previous study has also demonstrated that maternal exercise inhibits apoptotic cell death in developing Hsp70.1 KO mouse brain via up-regulation of Bcl-2, Hsp27 and 110, and down-regulation of caspases and Bax (Lee et al., 2011). However, further study is needed to evaluate the exact mechanisms of lower expression of Hsp27 in Hsp70.1 KO mice.

Our study demonstrated that the expression of Cu- and Mn-SOD, Bcl-2 and -XL, and Hsp27 was decreased after renal ischemia, and that of 4-HNE and caspases was increased in the Hsp70.1 KO mice; however, exercise preconditioning up-regulates antioxidant enzymes and anti-apoptotic factors and Hsp27 and significantly reduces 4-HNE and serum creatinine, a marker of glomerular filtration rate. It has been reported previously that exercise preconditioning before ischemia protects against damage to the brain and heart in experimental animals (Ding et al., 2006). Although the mechanisms underlying prevention of ischemic renal injury by exercise preconditioning are not clearly

understood yet, our findings suggest that exercise preconditioning may be a possible method of protecting the kidney from ischemic injury even under Hsp70 deficient circumstance.

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