Orchiectomy Attenuates Post-ischemic Oxidative Stress and Ischemia/Reperfusion Injury in Mice

A ROLE FOR MANGANESE SUPEROXIDE DISMUTASE

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Jinu Kim‡, †In Sup Kil†,§ Young Mi Seok‡, Eun Sun Yang‡, Dae Kyong Kim‡, Dong Gun Lim¶, Jeen-Woo Park†, Joseph V. Bonventre**§, and Kwon Moo Park†‡

From the Departments of Anatomy and Anesthesiology and Pain Medicine, School of Medicine, and the Department of Biochemistry, College of Natural Sciences, Kyungpook National University, Daegu 700-422, Korea. †Department of Environmental and Health Chemistry, College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea, and **Renal Division, Brigham and Women’s Hospital and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115

Males are much more susceptible to ischemia/reperfusion (I/R)-induced kidney injury when compared with females. Recently we reported that the presence of testosterone, rather than the absence of estrogen, plays a critical role in gender differences in kidney susceptibility to I/R injury in mice. Although reactive oxygen species and antioxidant defenses have been implicated in I/R injury, their roles remain to be defined. Here we report that the orchitectomized animal had significantly less lipid peroxidation and lower hydrogen peroxide levels in the kidney 4 and 24 h after 30 min of bilateral renal ischemia when compared with intact or dihydrotestosterone-treated orchitectomized males. The post-ischemic kidney expression and activity of manganese superoxide dismutase (MnSOD) in orchitectomized mice was much greater than in intact or dihydrotestosterone-administered orchitectomized mice. Four hours after 30 min of bilateral ischemia, superoxide formation was significantly lower in orchitectomized mice than in intact mice. In Madin-Darby canine kidney cells, a kidney epithelial cell line, 1 mM H2O2 decreased MnSOD activity, an effect that was potentiated by pretreatment with dihydrotestosterone. Orchiectomy prevented the post-ischemic decrease of catalase activity. Treatment of male mice with manganese(III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP), a SOD mimic, reduced the post-ischemic increase of plasma creatinine, lipid peroxidation, and tissue hydrogen peroxide. These results suggest that orchitectomy accelerates the post-ischemic activation of MnSOD and reduces reactive oxygen species and lipid peroxidation, resulting in reduced kidney susceptibility to I/R injury.

Gender differences in disease susceptibility have been well characterized in many cardiovascular diseases (1–3). The increased risk for cardiovascular diseases in men and postmenopausal women (4) has been attributed primarily to lack of estrogen-mediated protection (5–7). Recently we demonstrated that male mice are much more susceptible to kidney ischemia/reperfusion (I/R) injury when compared with female mice (8), and that orchiectomy decreases kidney susceptibility to I/R injury (9), whereas ovariectomy has no effect on kidney susceptibility to I/R injury (8). Testosterone administration to orchitectomized male or female mice reverses the protective phenotype, increasing susceptibility to kidney I/R injury. We concluded that the presence of testosterone, rather than the absence of estrogen, plays a critical role in the gender differences in kidney susceptibility to I/R injury. The detailed molecular mechanisms responsible for this testosterone effect remain to be defined.

Ischemia/reperfusion markedly increases the production of reactive oxygen species (ROS) including superoxide anions, hydroxyl radicals, hypochlorous acid, hydrogen peroxide, and peroxynitrite (10). The abnormal excessive production of ROS results in lipid peroxidation, leukocyte activation, endothelial cell damage, and cytokine production, all of which contribute to tissue damage (11–13). Reactive oxygen species disrupt the cellular cytoskeleton and cellular integrity and break down DNA (11–13). A number of studies have demonstrated that the activation of antioxidant enzymes or treatment with antioxidant agents ameliorates I/R injury (11, 14–17).

There are gender differences in the oxidant-antioxidant systems (18–21). Base-line oxidation is greater in males than in females (22). Estrogen has antioxidant properties at pharmacological concentrations, whereas testosterone does not (23). We hypothesized that testosterone may inhibit antioxidant defense systems, resulting in increased susceptibility to kidney I/R injury.

Here we report that orchitectomy accelerates the post-ischemic activation and expression of MnSOD in the kidney, leading to reduced superoxide radical levels and lipid peroxidation.

**The abbreviations used are: I/R, ischemia/reperfusion; MDCK, Madin-Darby canine kidney cells; ROS, reactive oxygen species; MDA, malondialdehyde; NBT, nitro blue tetrazolium; DHT, dihydroxytestosterone; SOD, superoxide dismutase; MnTMPyP, manganese(III) tetrakis(1-methyl-4-pyridyl)porphyrin.

1 To whom correspondence should be addressed: Dept. of Anatomy, School of Medicine, Kyungpook National University, 101 Dongin-2Ga, Jung-Gu, Daegu, 700-422, Korea. Tel.: 82-53-420-4804; Fax: 82-53-427-1468; E-mail: kpmpark@knu.ac.kr.

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3 Supported by NIDDK, National Institutes of Health Grant DK-39773.

4 To whom correspondence should be addressed: Dept. of Anatomy, School of Medicine, Kyungpook National University, 101 Dongin-2Ga, Jung-Gu, Daegu, 700-422, Korea. Tel.: 82-53-420-4804; Fax: 82-53-427-1468; E-mail: kpmpark@knu.ac.kr.
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effects that can explain the attenuated susceptibility of the kidney of the orchiectomized mouse to ischemic injury. Consistent with this is the finding that manganese(III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP), a mimic of SOD, reduces kidney susceptibility to I/R injury. Thus, the increase in antioxidant defenses associated with orchiectomy may account for reduced susceptibility to ischemic injury in orchiectomized mice.

MATERIALS AND METHODS

Animal Preparation—Experiments were performed in 12–14-week-old BALB/c male mice. In all the cases, studies were done according to animal experimental procedures approved by the Animal Care and Use Committee of Kyungpook National University. Each animal group consisted of more than four mice. Blood was collected to determine base line plasma creatinine concentrations.

Animals were anesthetized with pentobarbital sodium (60 mg/kg body weight; intraperitoneally) prior to surgery. Kidney ischemia was carried out as described previously (24, 25). In some animals, either orchiectomy or sham orchiectomy was carried out 15 days before either 30 min of bilateral renal ischemia or sham surgery. Some animals were administered dihydrotestosterone (DHT; 500 µg/kg body weight, Sigma), a non-aromatizable analogue of testosterone or vehicle (sesame oil, Sigma) by subcutaneous injection every day for 14 days prior to ischemia. Some animals were administered intraperitoneally either MnTMPyP (5 mg/kg body weight), a SOD mimetic, or vehicle (saline) for 12 days and then subjected to either 30 min of bilateral renal ischemia or sham operation. Kidneys were harvested for Western blot analysis and biochemical studies and frozen using liquid nitrogen. For the determination of superoxide formation, kidneys were frozen-fixed in liquid nitrogen and cut into 4-µm sections using a cryomicrotome (Cryotome). Sections were incubated in 1 mg/ml NBT (Sigma) in phosphate-buffered saline containing 0.1% Triton X-100, disrupted twice using a sonicator (4710 series; Cole-Parmer, Chicago, IL) at 40% of maximum setting for 10 s, and centrifuged at 15,000 × g for 30 min. The supernatant was used to measure the activities of manganese superoxide dismutase (MnSOD).

Measurement of Catalase, CuZnSOD, and MnSOD Activities in Kidney and MDCK Cells—Catalase activity was measured by the decomposition of hydrogen peroxide, determined by a decrease in absorbance at 240 nm. SOD activity was assayed spectrophotometrically using a pyrogallol assay as described previously (27). MnSOD or CuZnSOD activity was measured using the mitochondrial or cytosolic fraction, respectively. One unit of SOD activity was defined as the quantity of enzyme that reduces the superoxide-dependent color change by 50%. MDCK cells were grown in the phenol red-free Dulbecco’s modified Eagle’s medium containing 10% charcoal-deprived fetal bovine serum. Cells were pretreated in serum-free medium for 24 h, incubated with serum-free medium containing various concentrations of DHT for 24 h, and treated with either 1 mM H2O2 or vehicle.

Immunoblot Analyses—Immunoblot analyses were performed as described previously (24, 25) with anti-MnSOD or β-actin antibodies (Calbiochem).

Determination of Superoxide Formation Using Nitro Blue Tetrazolium (NBT)—Kidneys were harvested either at 4 h after sham operation or 30 min of bilateral renal ischemia. The kidneys were frozen-fixed in liquid nitrogen and cut into 4-µm sections using a cryomicrotome (Cryotome). Sections were incubated in 1 mg/ml NBT (Sigma) in phosphate-buffered saline, incubated with serum-free medium containing 10% charcoal-deprived fetal bovine serum. Cells were pretreated in serum-free medium for 24 h, incubated with serum-free medium containing various concentrations of DHT for 24 h, and treated with either 1 mM H2O2 or vehicle.

Preparation of Kidney Cytosolic and Mitochondrial Fractions—Kidneys in sucrose buffer (0.32 M sucrose, 10 mM Tris-HCl, pH 7.4) on ice were homogenized with a Dounce homogenizer. The homogenate was centrifuged at 1,000 × g for 5 min, and the supernatant was centrifuged at 15,000 × g for 30 min. The supernatant was used to measure the activity of catalase or copper-zinc superoxide dismutase (CuZnSOD). The precipitate was washed twice with sucrose buffer to collect mitochondrial pellets. The mitochondrial pellets were suspended in phosphate-buffered saline containing 0.1% Triton X-100, disrupted twice using a sonicator (4710 series; Cole-Parmer, Chicago, IL) at 40% of maximum setting for 10 s, and centrifuged at 15,000 × g for 30 min. The supernatant was considered statistically significant at a p value of 0.05 versus their respective sham ischemia controls. b, p < 0.05 versus 4 h after ischemia in intact/vehicle. c, p < 0.05 versus 4 h after ischemia in sham orchiectomy/vehicle. d, p < 0.05 versus 4 h after ischemia in orchiectomy/DHT. e, p < 0.05 versus 24 h after ischemia in intact/vehicle. f, p < 0.05 versus 24 h after ischemia in orchiectomy/DHT.
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Saline, pH 7.4, for 2 h at 37 °C and washed with phosphate-buffered saline. Signals were observed using a light microscope. The area of nitro blue tetrazolium positivity was analyzed using the image acquisition and analysis software, LabWorks (Ultra-Violet Products Ltd.).

Statistics—The results were expressed as mean ± S.E. Statistical differences among groups were calculated using analysis of variance followed by least significance difference post hoc comparison using the SPSS 12.0 program. Differences between groups were considered statistically significant at a p value of <0.05.

RESULTS

Orchiectomy Reduces Kidney Susceptibility to Ischemia/Reperfusion Injury in Mice—Thirty minutes of bilateral renal ischemia in intact mice markedly increased the plasma creatinine level at 4 and 24 h after reperfusion (Fig. 1). By contrast, this period of ischemia in orchiectomized mice did not result in the increase of plasma creatinine 4 and 24 h after ischemia (Fig. 1). To determine whether the reduced susceptibility of orchiectomized mice to ischemia is associated with testosterone, we induced ischemia in orchiectomized mice that had been pretreated with DHT. In these mice, post-ischemic plasma creatinine levels were similar to those in intact males (Fig. 1).

Ischemia/Reperfusion-induced Kidney Lipid Peroxidation and Production of Hydrogen Peroxide Were Reduced in Orchiectomized Mice—Thirty minutes of bilateral renal ischemia followed by 4 or 24 h reperfusion significantly increased lipid peroxidation and tissue hydrogen peroxide levels (Fig. 2A). The lipid peroxidation in intact males was much greater than in orchiectomized males 4 and 24 h after I/R (Fig. 2A). Tissue hydrogen peroxide levels were significantly lower in orchiectomized mice when compared with that in intact mice 4 and 24 h after I/R (Fig. 2B). This suggests that the reduced susceptibility to I/R injury in orchiectomized mice may be related to a reduction of ROS.

Activity of Catalase in Kidneys Subjected to Ischemia/Reperfusion—In intact males, 30 min of bilateral renal ischemia significantly decreased the kidney catalase activity 24 h after reperfusion, whereas in orchiectomized mice it did not (Fig. 3). To examine whether the reduced activity of catalase in intact males is associated with the levels of androgen, we determined the post-ischemic catalase activity in orchiectomized mice administered 500 μg/kg DHT. Twenty-four hours after ischemia, catalase activities in DHT-treated orchiectomized mice were significantly lower than sham-operated control (Fig. 3). We reported previously that orchiectomy decreases plasma testosterone, and DHT treatment increases plasma testosterone levels to those of the intact male (8).

Activity of CuZnSOD and MnSOD in Kidneys Subjected to Ischemia—Four and 24 h after ischemia, the activity of CuZnSOD in the cytosolic fraction of kidney tissues was not significantly changed in any of the three experimental animal groups when compared with their respective sham-operated control mice (Fig. 4A). Because MnSOD is a major antioxidant in mitochondria and is essential for life (28, 29), we determined the activity of MnSOD in the mitochondrial fraction of the kidneys. In intact males, ischemia significantly decreased MnSOD activity 24 h after ischemia (Fig. 4B). In orchiectomized males, however, ischemia did not attenuate the activity of MnSOD 24 h after reperfusion (Fig. 4B). In fact, MnSOD activity was increased 4 h after ischemia in orchiectomized males (Fig. 4B). Administration of DHT to orchiectomized mice prevented the increase (Fig. 4B). The increased MnSOD activity in orchiectomized mice was associated with an increase in total MnSOD protein in kidneys 4 h after reperfusion (Fig. 5). By contrast,
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Activity and Expression of MnSOD in MDCK Cells after Treatment with DHT or H$_2$O$_2$—Because epithelial cell injury is an important component of ischemia/reperfusion-induced injury in vivo, we determined the effect of DHT on the activity and expression of MnSOD in MDCK cells, an established kidney epithelial cell line. Treatment with DHT alone did not alter MnSOD expression and activity (Fig. 6A). One mM H$_2$O$_2$ treatment significantly decreased MnSOD activity in the mitochondrial fraction of the cells (Fig. 6B). Pretreatment with DHT resulted in significantly greater reductions in MnSOD activity after treatment with 1 mM H$_2$O$_2$ (Fig. 6B).

Orchectomy Reduces Post-ischemic Superoxide Formation in the Kidneys Subjected to Ischemia—Because superoxide produces blue formazan after reaction with nitro blue tetrazolium, we examined the deposition of blue formazan in kidney sections. Four hours after I/R, the formation of blue formazan dramatically increased in the tubular cells of the kidneys in intact mice (Fig. 7A). Superoxide formation was less in orchiectomized mice than it is in intact mice 4 h after I/R (Fig. 7A). The production of formazan was quantitated, and data are presented in Fig. 7B. This suggests that the higher activity of antioxidant enzymes such as MnSOD in orchiectomized mice reduces the amount of superoxide radicals leading to less susceptibility of the kidney to I/R injury.

MnTMPyP, a SOD Mimetic, Reduces Post-ischemic Lipid Peroxidation and Protects the Kidney from Ischemia—To determine whether the increased activation of MnSOD mediates the lower susceptibility of orchiectomized males to ischemia, we examined the effect of MnTMPyP, a mimetic of SOD, on I/R injury. Treatment with MnTMPyP for 12 days did not change body weight (data not shown). At 24 h after ischemia, plasma creatinine concentration significantly increased in vehicle-treated mice, whereas ischemia resulted in only modest increases in MnTMPyP-treated mice (Fig. 8). To examine whether the protection afforded by MnTMPyP was associated with reduced oxidative stress, we determined the lipid peroxidation and the tissue hydrogen peroxide levels in the kidney 4 and 24 h after reperfusion. Prior administration of MnTMPyP resulted in significant reductions in post-ischemic lipid peroxidation and tissue hydrogen peroxide levels when compared with vehicle-treated animals (Fig. 9). Catalase activity was not affected.
by MnTMPyP administration and decreases 24 h after ischemia in both vehicle- and MnTMPyP-treated mice (Fig. 10A). Pretreatment with MnTMPyP did not affect the activities of CuZnSOD 4 and 24 h after ischemia (Fig. 10B). MnTMPyP prevented the post-ischemic reduction of MnSOD activity (Fig. 10C).

**DISCUSSION**

Our findings build on our previous data that removal of the testes in males reduces kidney susceptibility to ischemia/reperfusion injury and that the reduced susceptibility associated with orchiectomy is reversed by addition of exogenous testosterone. We have now found a mechanism of this important gender difference in susceptibility to ischemia. The protection afforded by orchiectomy is related to a reduction of oxidative stress as reflected by reduced lipid peroxidation and reduced tissue levels of hydrogen peroxide and superoxide formation with greater MnSOD activity in the kidneys of orchiectomized mice. We also demonstrate that MnTMPyP, a SOD mimetic, reduces the post-ischemic lipid peroxidation and renal functional impairment in mice. Although many studies have emphasized the beneficial effect of estrogen in gender differences in ischemia/reperfusion injury, this study, together with our prior observations, demonstrates that male sex hormones play an important role in the gender difference in kidney susceptibility to I/R injury (8, 30).

Excessive ROS production is an important cause of I/R-induced injury (11, 32). ROS have been implicated in gender
differences in diseases (33, 34). Borras et al. (18) reported that females have higher antioxidant enzymes resulting in the lower production of ROS than in males and that orchiectomy increases the production of ROS. In postmenopausal women with coronary heart disease, testosterone concentration and MDA production, an indicator of lipid peroxidation, are elevated in serum when compared with values obtained in normal healthy women (34). By contrast, SOD and estradiol levels are significantly lower than in normal healthy women (34). Strehlow et al. (33) reported that 17β-estradiol up-regulates MnSOD and extracellular superoxide dismutase expression and enzyme activity in vascular smooth muscle cells.

Lipid peroxidation and production of ROS are regulated by various extracellular and intracellular antioxidant enzymes. A contributing factor to the increase of ROS levels after I/R is the reduction of antioxidant enzyme activity. It has been reported that catalase activity is decreased after I/R (11). In the present study, the activity of catalase, which transforms H₂O₂ to H₂O, was significantly decreased after ischemia in the intact mice, whereas orchiectomy prevented the post-ischemic decreases in catalase activity. In addition, DHT administration to orchiectomized mice prevented the effects of orchietomy on the post-ischemic decrease of catalase activity, indicating that testosterone plays an important role in the regulation of antioxidant enzyme activity. H₂O₂ production was greater in intact males than in orchiectomized mice.

Superoxide is an important free radical mediating cellular damage. It is scavenged by CuZnSOD located in cytoplasm and MnSOD in mitochondria. MnSOD is the major antioxidant enzyme located in mitochondria and is essential for life (28, 29). Chien et al. (37) reported that intravenous treatment with SOD in rats results in reduced apoptotic cell death and ROS production after kidney ischemia/reperfusion. Macmillan-Crow and Cruthirds (35) reported that mice expressing only 50% of the normal complement of MnSOD demonstrate increased susceptibility to oxidative stress and severe mitochondrial dysfunction resulting from elevation of ROS (35). On the other hand, overexpression of MnSOD enzyme renders the cells and tissues resistant to ischemia/reperfusion injury (36). In the present study, orchietomy resulted in enhanced post-ischemic MnSOD, but not CuZnSOD, activity and expression, resulting in the reduction of post-ischemia superoxide radical formation. Thus the higher ROS stress in intact males may be directly related to lower MnSOD activity (37).

To test whether testosterone alone suppresses the expression and activity of MnSOD by a direct effect on the epithelial cell,
we evaluated whether DHT modulates MnSOD activity and expression in MDCK cells, a well established tubular epithelial cell line. DHT treatment alone did not affect the expression and activity of MnSOD in these cells. Thirty or sixty minutes after treatment with 1 mM H$_2$O$_2$, however, MnSOD activity was significantly lower in DHT-pretreated MDCK cells than that in vehicle-pretreated cells (Fig. 6B). This indicates that the post-ischemic suppression of MnSOD activity in DHT-treated orchietomized mice may reflect, at least in part, a direct effect on the epithelial cell.

MnSOD is susceptible to nitrotyrosylation, which resulted in enzyme inactivation (38, 39). In the present study, ischemia in intact male mice decreases MnSOD activity without a significant decrease of MnSOD expression. We speculate therefore that MnSOD is inactivated by nitration. MacMillan-Crow et al. (38, 39) demonstrated in chronic renal allograft rejection that MnSOD is endogenously tyrosine nitrated and inactivated. Crucifers et al. (40) reported that I/R in kidney decreases MnSOD activity without a reduction in MnSOD expression, and this decrease of MnSOD activity is associated with an increase in MnSOD nitration. Sam et al. (41) reported that in human hearts there can be a dissociation between mRNA expression and protein abundance or activity of catalase and MnSOD.

ROS production by mitochondria from females is much lower than that from males (18), indicating that levels of sexual hormones affect MnSOD activity. In the present study, although the treatment of orchietomized mice with DHT restored kidney susceptibility from a resistant to susceptible phenotype, the post-ischemic reduction of MnSOD activity was not restored. This may be caused by subtle differences of hormone levels between intact and DHT-treated orchietomized mice.

Thirty minutes of bilateral ischemia in non-orchietomized mice results in the disruption of most of tubular cells in the outer medulla leading to kidney dysfunction (8, 24, 25, 42), whereas the same period of ischemia in orchietomized mice does not induce extensive cellular death. In the present study, 30 min of ischemia in orchietomized mice produced small amounts of free radicals as seen in Fig. 7 without extensive tubular cell death, lipid peroxidation, and kidney functional impairment (8). Orchiectomy alone did not modulate superoxide formation. Differences in oxidants and antioxidant enzymes related to orchietomy only emerged after an ischemic challenge.

MnTMPyP is a SOD mimetic that protects the heart from ischemia/reperfusion injury (31). In the present study, MnTMPyP reduced the post-ischemic lipid peroxidation and the tissue levels of hydrogen peroxide in the kidney. These results support the hypothesis that the increased MnSOD activity in orchietomized mice may account for the reduced susceptibility associated with orchietomy. Recently we also reported that MnTMPyP protects cells from oxidative stress associated with radiation (26).

In summary, our data indicate that removal of testes reduces kidney susceptibility to ischemia/reperfusion injury and results in decreased lipid peroxidation, increased post-ischemic catalase, and MnSOD activities. The higher activity of antioxidant enzymes may account for the lower susceptibility of orchietomized mice to ischemic injury. Our findings may have important implications for understanding the pathophysiology of gender differences in kidney ischemia/reperfusion injury and should lead to consideration of androgens and the ROS-antioxidant systems as targets for protective strategies to avoid acute kidney injury.

REFERENCES
1. Reckelhoff, J. F., Zhang, H., Srivistava, K., and Granger, I. P. (1999) Hypertension 34, 920–923
2. Silbiger, S. R., and Neugarten, J. (1995) Am. J. Kidney Dis. 25, 515–533
3. Silbiger, S. R., and Neugarten, J. (2003) Adv. Renal. Replace. Ther. 10, 3–14
4. Reckelhoff, J. F., and Granger, I. P. (1999) Clin. Exp. Pharmacol. Physiol. 26, 127–131
5. Squadrato, F., Altavilla, D., Squadrato, G., Campo, G. M., Arlotto, M., Arcoraci, V., Minutoli, L., Serrano, M., Saitta, A., and Caputi, A. P. (1997) Eur. J. Pharmacol. 335, 185–192
6. Camper-Kirby, D., Welch, S., Walker, A., Shiraishi, I., Setchell, K. D., Schafer, E., Kajstura, J., Anversa, P., and Sussman, M. A. (2001) Circ. Res. 88, 1020–1027
7. Fukuda, K., Yao, H., Ibayashi, S., Nakahara, T., Uchimura, H., Fujishima, M., and Hall, E. D. (2000) Stroke 31, 155–160
8. Park, K. M., Kim, J. I., Ahn, Y., Bonventre, A. J., and Bonventre, J. V. (2004) J. Biol. Chem. 279, 52282–52292
9. Park, K. M., Cho, H. J., and Bonventre, J. V. (2005) Biochem. Biophys. Res. Commun. 328, 312–317
10. Kaminski, K. A., Bonda, T. A., Korecki, J., and Musial, W. J. (2002) Int. J. Cardiol. 86, 41–59
11. Dobashi, K., Ghosh, B., Orak, J. K., Singh, L., and Singh, A. K. (2000) Mol. Cell. Biochem. 205, 1–11
12. Bonventre, J. V. (1993) Kidney Int. 43, 1160–1178
13. Toyokuni, S. (1999) Pathol. Int. 49, 91–102
14. Eltzschig, H. K., and Collard, C. D. (2004) Br. Med. Bull. 70, 71–86
15. Land, W., Schneebberger, H., Schleibner, S., Illner, W. D., Abendroth, D., Rutili, G., Arfors, K. E., and Messmer, K. (1994) Transplantation 57, 211–217
16. Gurel, A., Armutcu, F., Sahin, S., Sogut, S., Ozuyurt, H., Gulec, M., Kutlu, N. O., and Akyol, O. (2004) Clin. Chim. Acta 339, 33–41
17. Inal, M., Altinmisik, M., and Bilgin, M. D. (2002) Cell Biochem. Funct. 20, 291–296
18. Borras, C., Sastre, J., Garcia-Sala, D., Lloret, A., Pallardo, F. V., and Vina, J. (2003) Free Radic. Biol. Med. 34, 546–552
19. Arnal, J. F., Clamens, S., Pechet, C., Negre-Salvayre, A., Allera, C., Girolami, J. P., Salvayre, R., and Bayard, F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4108–4113
20. Ruiz-Larrea, M. B., Leal, A. M., Martin, C., Martinez, R., and Lacort, M. (1997) Rev. Esp. Fisiol. 53, 225–229
21. Ruiz-Larrea, B., Leal, A., Martin, C., Martinez, R., and Lacort, M. (1995) Steroids 60, 780–783
22. Actis-Gorellata, L., Carrasquedo, F., and Fraga, C. G. (2004) Clin. Chim. Acta 349, 97–103
23. Mooradian, A. D. (1993) J. Steroid Biochem. Mol. Biol. 45, 509–511
24. Park, K. M., Chen, A., and Bonventre, J. V. (2001) J. Biol. Chem. 276, 11870–11875
25. Park, K. M., Kramers, C., Vayssier-Taussat, M., Chen, A., and Bonventre, J. V. (2002) J. Biol. Chem. 277, 2040–2049
26. Lee, J. H., and Park, J. W. (2004) Free Radic. Biol. Med. 37, 272–283
27. Martindale, S., and Marklund, G. (1974) Eur. J. Biochem. 47, 469–474
28. Li, Y., Huang, T. T., Carlson, E. J., Melov, S., Ursell, P. C., Olson, J. L., Noble, L. J., Yoshimura, M. P., Berger, C., and Chan, P. H. (1995) Nat. Genet. 11, 376–381
29. Lebovitz, R. M., Zhang, H., Vogel, H., Cartwright, J., Jr., Dionne, L., Lu, N., Huang, S., and Matzuk, M. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9782–9787
30. Muller, V., Losonczy, G., Heemann, U., Vannay, A., Fekete, A., Reusz, G., Tulassay, T., and Szabo, A. J. (2002) Kidney Int. 62, 1364–1371
31. Jain, M., Cui, L., Brenner, D. A., Wang, B., Handy, D. E., Leopold, J. A., Loscalzo, J., Apstein, C. S., and Liao, R. (2004) Circulation 109, 898–903
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32. Aragno, M., Cutrin, J. C., Mastrocola, R., Perrelli, M. G., Restivo, F., Poli, G., Danni, O., and Boccuzzi, G. (2003) Kidney Int. 64, 836–843
33. Strehlow, K., Rotter, S., Wassmann, S., Adam, O., Grohe, C., Laufs, K., Bohm, M., and Nickenig, G. (2003) Circ. Res. 93, 170–177
34. Deng, H., Zhou, H., and Sun, M. (1999) Hunan Yi Ke Da Xue Xue Bao 24, 343–346
35. Macmillan-Crow, L. A., and Cruthirds, D. L. (2001) Free Radic. Res. 34, 325–336
36. Chen, Z., Siu, B., Ho, Y. S., Vincent, R., Chua, C. C., Hamdy, R. C., and Chua, B. H. (1998) I. Mol. Cell. Cardiol. 30, 2281–2289
37. Chien, C. T., Lee, P. H., Chen, C. F., Ma, M. C., Lai, M. K., and Hsu, S. M. (2001) J. Am. Soc. Nephrol. 12, 973–982
38. MacMillan-Crow, L. A., Cruthirds, D. L., Akhi, K. M., Sanders, P. W., and Thompson, J. A. (2001) Free Radic. Biol. Med. 31, 1603–1608
39. MacMillan-Crow, L. A., Crow, J. P., Kerby, J. D., Beckman, J. S., and Thompson, J. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11853–11858
40. Cruthirds, D. L., Novak, L., Akhi, K. M., Sanders, P. W., Thompson, J. A., and MacMillan-Crow, L. A. (2003) Arch. Biochem. Biophys. 412, 27–33
41. Sam, F., Kerstetter, D. L., Pimental, D. R., Mulukutla, S., Tabae, A., Bris-tow, M. R., Colucci, W. S., and Sawyer, D. B. (2005) J. Card. Fail. 11, 473–480
42. Park, K. M., Byun, J. Y., Kramers, C., Kim, J. I., Huang, P. L., and Bonventre, J. V. (2003) J. Biol. Chem. 278, 27256–27266