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Protective role of cytosolic NADP$^+$-dependent isocitrate dehydrogenase, IDH1, in ischemic pre-conditioned kidney in mice

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Abstract

Ischemic pre-conditioning protects the kidney against subsequent ischemia/reperfusion (I/R). This study investigated the role of cytosolic NADP$^+$ -dependent isocitrate dehydrogenase (IDH1), a producer of NADPH, in the ischemic pre-conditioning. Mice were pre-conditioned by 30 min of renal ischemia and 8 days of reperfusion. In non-pre-conditioned mice 30 min of ischemia had significantly increased the levels of plasma creatinine, BUN, lipid peroxidation and hydrogen peroxide in kidneys, whereas in pre-conditioned mice, the ischemia did not increase them. The reductions of reduced glutathione and NADPH after I/R were greater in non-pre-conditioned mice than in pre-conditioned mice. Ischemic pre-conditioning prevented the I/R-induced decreases in IDH1 activity and expression, but not in glucose-6-phosphate dehydrogenase activity. In conclusion, protection of the kidney afforded by ischemic pre-conditioning may be associated with increased activity of IDH1 which relates to increased levels of NADPH, increased ratios of GSH/total glutathione, less oxidative stress and less kidney injury induced by subsequent I/R insult.

Keywords: *NADPH, glutathione, GSH, isocitrate dehydrogenase, G6PD, acute kidney injury*

Introduction

Organs previously exposed to an ischemia and reperfusion (I/R) insult are resistant to subsequent I/R insults. The phenomenon, called ischemic pre-conditioning, has been demonstrated in a number of organs including the kidney [1–8]. Recently, we have reported that ischemic pre-conditioning by a single event of ischemia and reperfusion renders the kidney resistant to subsequent I/R insults; the protection by ischemic pre-conditioning is highly effective and persists for at least 12 weeks [7,8]. However, the molecular mechanism of protection remains to be elucidated.

I/R in tissues including kidney increases the levels of reactive oxygen species (ROS) by the activation of ROS-producing systems and simultaneous impairment of antioxidant scavenging enzymes [9]. Excessive production of ROS after I/R causes lipid peroxidation, protein degradation and DNA breakdown, resulting in tissue damage [9–13]. Accumulating evidence has demonstrated that ROS production and scavenging are critical modulators for the degree of I/R injury in various organs [9,14–16].

NADPH is an essential co-factor for the generation of reduced glutathione (GSH) [17,18], which is the most abundant low molecular-mass thiol in mammalian cells [19,20]. In addition, NADPH is used for the conversion of oxidized thioredoxin to reduced thioredoxin, which also plays a role as an antioxidant [21,22]. The NADPH is produced by at least four enzymes: glucose-6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase, malate dehydrogenase and NADP$^+$-dependent isocitrate dehydrogenases (IDHs) [23–25]. NADP$^+$-dependent isocitrate dehydrogenases consist of IDH1 which is expressed in the cytosol and IDH2 which is found in the mitochondria [23,26]. Recently, we found that renal I/R

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reduced the activity and expression of IDH1 along with a decrease in NADPH production in the kidney [27]. Expression and activity of IDH1 differs within various regions of the kidney. For example expression of IDH1 is low in the S3 segment of the proximal tubule which is most sensitive to I/R-induced injury [27]. Up-regulation of IDH1 expression in cultured kidney epithelial cells decreases cell susceptibility to oxidative stress, whereas down-regulation of IDH1 expression renders cells more vulnerable to insult [27]. It has been reported that G6PD is a primary source of NADPH in various cells [21]. However, we recently found that the contribution of IDH1 for NADPH production is 10–30-times higher than G6PD in the kidney [27]. Additionally, IDH1 mRNA is highly expressed in the liver and kidney [28], suggesting that IDH1 is a major contributor for the generation of NADPH in the kidney and that IDH1 is tightly associated with kidney I/R injury.

Therefore, in the present study we investigated the relationship of NADPH and IDH1 in the ischemic pre-conditioning phenomenon. We found that the ischemic pre-conditioned mouse kidney was less susceptible to a subsequent I/R insult. Further, in those ischemic pre-conditioned kidneys, there was greater IDH1 activity, higher levels of NADPH and GSH along with less production of hydrogen peroxide, lipid production and preservation of renal function. These data suggest that IDH1 may be considered as a target protein for the development of therapeutics for I/R-induced kidney diseases including acute kidney injury (AKI) of which mortality and morbidity are very high and where effective therapeutics have not been developed yet [29,30].

Materials and methods

Animal preparation

All experiments were performed with 8–10-week-old C57BL/6 male mice obtained from Koatech (Kyungkido, Korea). The studies were performed under the guidelines of the Institutional Animal Care and Use Committee of Kyungpook National University. Mice were allowed free access to water and standard mouse chow. Mice were anaesthetized with pentobarbital sodium (60 mg/kg body weight; intraperitoneally; Sigma, St. Louis, MO) and subjected to bilateral renal ischemia as previously described [7,31]. During surgery, body temperature was maintained at 36.5–37.5°C. Some mice were pre-conditioned by 30 min of bilateral renal ischemia and 8 days of reperfusion. For control, mice were subjected to sham-operation instead of 30 min of ischemia. To evaluate the accuracy and strength of pre-conditioning plasma creatinine (PCr) levels were determined 24 h after the pre-conditioning. Pre-conditioned mice with low PCr concentration were not used for further I/R studies. After 8 days from the pre-conditioning or sham-operation (non-pre-conditioning), mice were subjected to either 30 min of bilateral renal ischemia or sham-operation. Mice were randomly assigned to I/R and sham-operation at the first surgeries (pre-conditioning and non-pre-conditioning) and the second surgeries (I/R and sham-operation).

Kidney preparation

To perform biochemical experiments, kidneys were snap-frozen in liquid nitrogen and kept in a deep-freezer until use.

Plasma creatinine and blood urea nitrogen (BUN) concentration

Seventy microlitres of blood were taken from the retrobulbar vein plexus using a heparinized-capillary glass tube at the times indicated in the figures. PCr and BUN concentrations were measured using the Vitros 250 (Johnson & Johnson, Rochester, NY, USA).

Measurement of lipid peroxidation and H_2O_2

To determine lipid peroxidation, kidney samples were evaluated for malondialdehyde (MDA) production using a spectrophotometric assay for thiobarbituric acid-reactive substances (TBARS). Briefly, kidneys were homogenized and 100 μg of kidney lysate was added in the 1.4 ml of reaction buffer (0.375% of thiobarbituric acid, 15% of trichloroacetic acid and 0.25 N HCl). The mixtures were boiled in a water bath for 15 min, cooled at room temperature, centrifuged at 12 000 rpm (Eppendorf, 5415C) for 10 min and then measured absorbance at 535 nm wavelength. H_2O_2 levels were measured using a ferric sensitive dye, xylenol orange. H_2O_2 oxidizes iron (II) to iron (III) in the presence of sorbitol, which acts as a catalyst. Iron (III) forms a purple complex with xylenol orange as previously described [9,32].

Western blot analysis

Western blot analyses were performed as described previously [7]. Briefly, renal tissue was homogenized in lysis buffer [7] containing inhibitors of proteinase and phosphatase and the lysates were centrifuged at 14 000 rpm (Eppendorf, 5415C) for 20 min at 4°C. Supernatant was collected and protein concentration was determined using the Bradford protein assay. Protein sample was mixed with an SDS-sample buffer and denatured by 5 min of boiling at 95°C. The protein sample was separated on 10% SDS-PAGE gels and then transferred to Immobilon membranes (Millipore Corp., Bedford, MA). The membrane was incubated with anti-IDH1 [33] antibody overnight at

4°C. After washing, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody, exposed to Western Lighting Chemiluminescence Reagent (NEL101; PerkinElmer LAS, Boston, MA) and then developed using X-ray film. The area of each band was analysed using LabWorks 4.5 software (UVP, Upland, CA).

Measurement of IDH1 and glucose-6-phophate dehydrogenase (G6PD) activity

The activities of both IDH1 and G6PD in kidney were measured as described previously [27,28,34]. Briefly, IDH1 activity in the cytosolic fraction (50 μg protein) was measured in the reaction mixture containing 40 mM Tris (pH 7.4), 2 mM $NADP^+$, 2 mM $MgCl_2$ and 50 mM threo-DS-isocitrate (Sigma). One unit of IDH1 activity was defined as the amount of enzyme catalysing the production of 1 μmol of NADPH/mi as measured by the absorbance at 340 nm at 37°C. G6PD activity in the cytosolic fraction (50 μg protein) was measured in the reaction mixture containing 55 mM Tris (pH 7.8), 3.3 mM $MgCl_2$, 240 μM $NADP^+$ and 4 mM glucose 6-phosphate (Sigma). One unit of G6PD activity was defined as the amount of enzyme catalysing the reduction of 1 μmol of $NADP^+$/min as measured by the absorbance at 340 nm at 37°C. The cytosolic fractionation was performed as described previously [27].

Measurement of cytosolic NADPH and total NADP ($NADP^+$ +NADPH) level

The cytosolic NADPH level was measured as described previously [27,28,35]. Briefly, NADPH in the cytosolic fraction was induced by exclusion of NADP through heating for 30 min at 60°C. Cytosolic fractions (200 μg protein, respectively) for NADPH and NADPt levels were pre-incubated in the reaction mixture containing 100 mM Tris (pH 8.0), 2 mM phenazine ethosulphate, 5 mM EDTA, 0.5 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 1.5 unit G6PD (Sigma) for 5 min at 37°C. The reactions were started by the addition of 1 mM glucose 6-phosphate. NADPH and total NADP ($NADP^+$ + NADPH) levels were defined as the change in optical density (O.D.) at 570 nm for 1 min at 37°C.

Measurement of ratio of oxidized glutathione (GSSG) to total glutathione

The ratio of oxidized glutathione (GSSG) to total glutathione (GSH + GSSG) was measured by an enzymatic recycling method as described previously [27,28,36]. The amount of total glutathione (GSH + GSSG) was determined by the formation of 5-thio-2-nitrobenzoic acid (TBA) converted from 5,5-dithiobis (2-nitrobenzoic acid) (DTNB). GSSG was measured by removing the TBA produced by the reaction of GSH and DTNB by adding of 2-vinylpyridine which inhibits the formation of TBA by GSH. Total glutathione and GSSG levels were defined as the change in OD at 412 nm for 1 min at 37°C.

Statistics

Results were presented as mean ± SEM. Statistical differences among groups were determined using analysis of variance (ANOVA) followed by least-significant 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

Ischemic pre-conditioning prevents I/R-induced increases in tissue H_2O_2, lipid peroxidation, plasma creatinine and BUN levels

H_2O_2 concentrations in kidneys of non-pre-conditioned mice were significantly increased at 4 and 24 h after ischemia (Figure 1A). However, H_2O_2 levels in kidneys of pre-conditioned mice remained unchanged. In agreement with H_2O_2 levels, lipid peroxidation levels in non-pre-conditioned mouse kidneys were significantly increased at 4 and 24 h after ischemia, whereas those in pre-conditioned mice were not (Figure 1B). Thirty minutes of ischemia in the non-pre-conditioned mouse, but not in the pre-conditioned mouse, significantly increased levels of PCr and BUN (Figures 2A and B). These results indicate that the protection of renal function by pre-conditioning is associated with lower I/R-induced oxidative stress. Sham-operation did not induce any significant changes in tissue H_2O_2 and lipid peroxidation levels in mice non-pre-conditioned when compared with concentrations in normal kidney tissue (Figures 1A and B), indicating that sham-operation did not induce any significant oxidative stress to the kidney.

Ischemic pre-conditioning prevents I/R-induced increases in levels of tissue GSSG/total glutathione

Thirty minutes of renal ischemia significantly increased the ratio of GSSG to total glutathione in kidney tissue of non-pre-conditioned mice 4 and 24 h after ischemia, whereas the ratio did not increase in kidneys of pre-conditioned-treated mice (Figure 3A). Furthermore, ischemia significantly reduced total glutathione levels in kidneys of non-pre-conditioned mice 4 and 24 h after the ischemia, whereas total glutathione was not effective in kidneys following

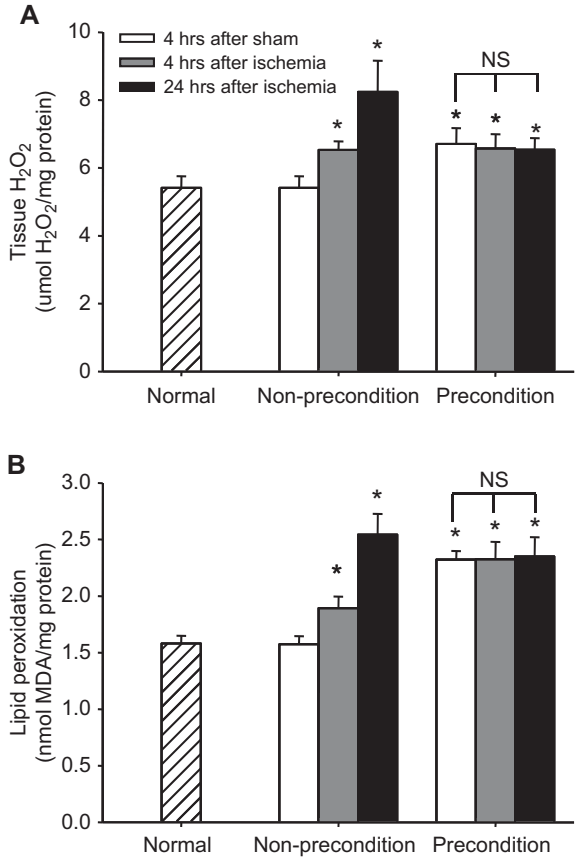

Figure 1. Levels of (A) hydrogen peroxide (H_2O_2) and (B) lipid peroxidation in kidney. Mice were pre-conditioned by either 30 min of bilateral renal ischemia (pre-condition) or sham-operation (non-pre-condition) on day 0 and 8 days later, they were subjected to another 30 min of bilateral renal ischemia or sham-operation. Kidneys were harvested 4 and 24 h after the surgeries. Levels of (A) H_2O_2 and (B) lipid peroxidation were determined in the kidneys. The results are presented as the means ± SE ($n = 6$). $^*p < 0.05$ vs 4 h after sham of non-pre-condition. NS, no statistical difference when compared with 4 h after S in pre-condition.

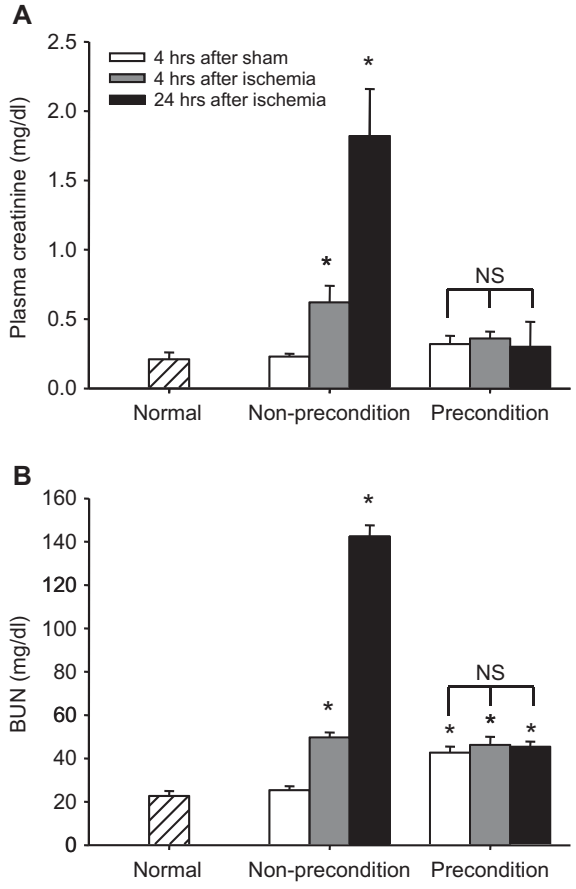

Figure 2. Concentrations of (A) plasma creatinine and (B) blood urea nitrogen (BUN). Mice were pre-conditioned by either 30 min of bilateral renal ischemia (pre-condition) or sham-operation (non-pre-condition) on day 0 and 8 days later, they were subjected to another 30 min of bilateral renal ischemia or sham-operation. Concentrations of (A) plasma creatinine and (B) plasma BUN were determined 4 and 24 h after the surgeries. The results are presented as means ± SE ($n = 6$). $^*p < 0.05$ vs 4 h after sham of non-pre-condition. NS, no statistical difference when compared with 4 h after S in pre-condition.

pre-conditioning (Figure 3B). Sham-operation in non-pre-conditioned mice did not induce any significant changes in the ratio of GSSG to total glutathione or total glutathione levels when compared with those in normal kidney tissue (Figures 3A and B).

Ischemic pre-conditioning prevents I/R-induced decreases in activity and expression of IDH1 after I/R

The conversion of GSSG to GSH is highly dependent on levels of NADPH, which is produced by IDHs and G6PD. IDH1 activity in kidneys of non-pre-conditioned mice was significantly decreased at 4 and 24 h after ischemia; however, it was not decreased in pre-conditioned mouse kidneys at the same time points (Figure 4A). Moreover, expression levels of IDH1 were significantly decreased after ischemia in kidneys of non-pre-conditioned mouse kidneys, whereas IDH1 was not decreased in kidneys after pre-conditioning (Figure 4B).

Ischemic pre-conditioning prevents I/R-induced decreases in the ratio NADPH of total NADP (NADPH/NADPt) after I/R

G6PD has long been considered as a primary source of NADPH [21] and it has been reported that G6PD gene deficiency increased myocardial dysfunction after I/R in mice [37]. Therefore, we determined the involvement of G6PD activity in our model Tissue G6PD activity was significantly decreased in tissue 24 h after ischemia in the non-pre-conditioned mouse, however activities did not decrease in the pre-conditioned mouse significantly (Figure 5). The ratio of NADPH to total NADP (NADPH/NADPt) in kidneys from the non-pre-conditioned mouse decreased 4 and 24 h after ischemia (Figure 6). However, the ratio of NADPH to total NADP in pre-conditioned mouse kidneys was even increased after ischemia (Figure 6). These results indicate that the preserved activity of IDH1 in kidneys of

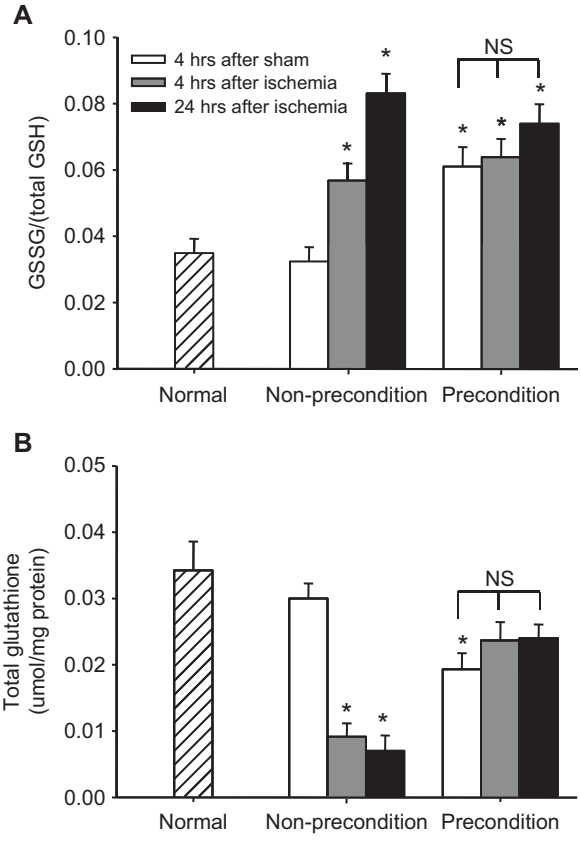

Figure 3. (A) Ratio of oxidized glutathione (GSSG) to total glutathione (GSSG + GSH) and (B) levels of total glutathione in kidney. Mice were pre-conditioned by either 30 min of bilateral renal ischemia (pre-condition) or sham-operation (non-pre-condition) on day 0 and 8 days later, they were subjected to 30 min of bilateral renal ischemia or sham-operation. Kidneys were harvested 4 and 24 h after the surgeries. (A) Ratio of GSSG/(GSH + GSSH) and level of total glutathione (B) were determined in the kidneys as described in the Materials and methods section. Results are expressed as means ± SE ($n = 6$). $^*p < 0.05$ vs 4 h after sham of non-pre-condition. NS, no statistical difference when compared with 4 h after S in pre-condition.

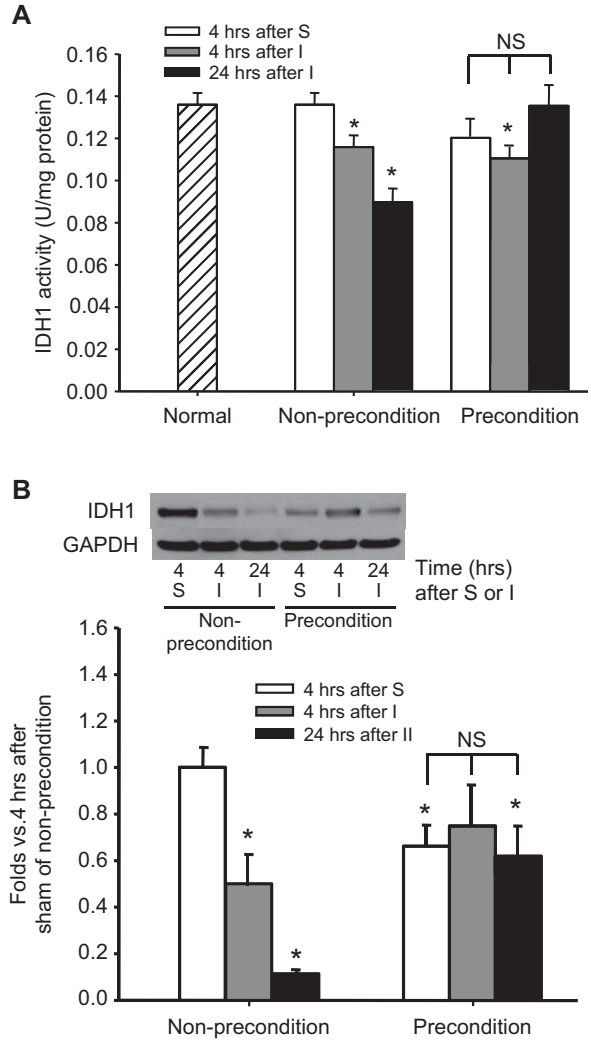

Figure 4. Activity (A) and expression (B and C) of IDH1 in kidneys. Mice were pre-conditioned by either 30 min of bilateral renal ischemia (pre-condition) or sham-operation (non-pre-condition) on day 0 and 8 days later, they were subjected to 30 min of bilateral renal ischemia (I) or sham-operation (S). Kidneys were harvested 4 and 24 h after the surgeries. (A) IDH1 activity was determined in the kidneys ($n = 6$). (B) IDH1 expression was determined by Western blot analysis using anti-IDH1 antibody. GAPDH expression was determined as an equal loading marker. The densities of blots were quantified using the Lab Works program ($n = 3$). Results are expressed as means ± SE. $^*p < 0.05$ vs 4 h after sham of non-precondition. NS, no statistical difference when compared with 4 h after S in pre-condition.

pre-conditioned mice after ischemia might increase NADPH production to protect the kidney from subsequent I/R injury.

Discussion

In the present study, we report that ischemic pre-conditioning protects kidneys against oxidative stress and renal functional impairment induced by I/R and the protection is associated with the increase of IDH1 activity, which leads to the increases of NADPH and GSH amounts. Because effective pharmacological regulators of IDH1 and genetically IDH1-gene modified animals have not been used in the present study, we could not directly prove the role of IDH1 as a mediator of protection afforded by ischemic pre-conditioning. However, our current data demonstrate that IDH1 is associated with ischemic pre-conditioning-induced protection through the serial activation of the IDH1-NADPH-GSH axis.

Although 8 days after pre-conditioning renal function returned to almost normal ranges, ischemic pre-conditioned kidneys presented higher levels of lipid peroxidation, H_2O_2, ratio of GSSG to total glutathione, whereas IDH1 expression and activity, G6PD and total glutathione were lower than non-pre-conditioned kidneys. Those changes in the pre-conditioned kidney may be due to the differentiation of survival or regenerated tubular epithelial cells, fibrotic changes, as well as inflammatory responses after I/R injury [8,38]. It has been reported that antioxidant systems are associated with the transforming status of cells and cell

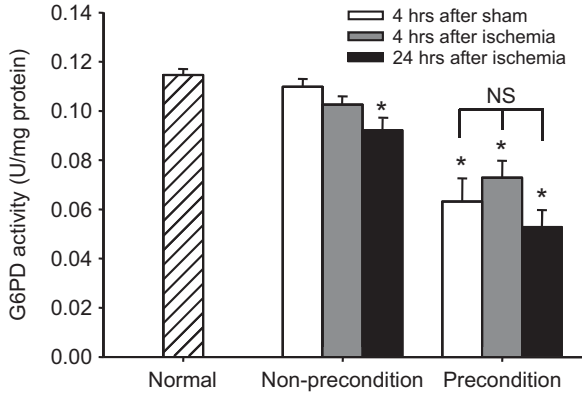

Figure 5. G6PD activity in kidneys. Mice were pre-conditioned by either 30 min of bilateral renal ischemia (pre-condition) or sham-operation (non-pre-condition) on day 0 and 8 days later, they were subjected to 30 min of bilateral renal ischemia or sham-operation. Kidneys were harvested 4 and 24 h after the surgeries and G6PD activity was determined in the kidneys. Results are expressed as means ± SE ($n = 6$). $^{*}p < 0.05$ vs 4 h after sham of non-pre-condition. NS, no statistical difference when compared with 4 h after S in pre-condition.

types [39,40].Glutathione is the most abundant low molecular weight thiol which plays an antioxidant role in tissues. Accumulating evidence has demonstrated that GSH prevents cells from oxidative stress, which is a major cause of I/R injury [10,41,42]. In the present study, I/R decreased total glutathione levels in non-pre-conditioned kidneys, but not in pre-conditioned kidneys. However, post-ischemic increase in the ratio of GSSG to total glutathione was prevented by ischemic pre-conditioning. These data suggest that the decrease of post-I/R oxidative tissue damage afforded by pre-conditioning is associated with higher GSH concentrations, rather than total glutathione concentrations, resulting from a greater conversion of GSSG to GSH. It is well known that the GSH attenuates renal cell damage against oxidative stress and I/R injury [41,42].

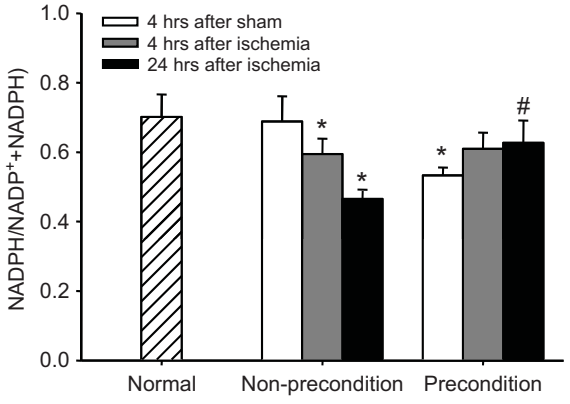

Figure 6. Ratio of NADPH to total NADP (NADPH/NADPt) in kidneys. Mice were pre-conditioned by either 30 min of bilateral renal ischemia (pre-condition) or sham-operation (non-pre-condition) on day 0 and 8 days later, they were subjected to 30 min of bilateral renal ischemia or sham-operation. Kidneys were harvested 4 and 24 h after the surgeries. NADPH/($NADP^+$ +NADPH) was determined in the kidneys as described in the Materials and methods section. Results are expressed as means ± SE ($n = 6$). $^{*}p < 0.05$ vs 4 h after sham of non-precondition. $^{\#}p < 0.05$ vs 4 h after sham of pre-condition.

Production of GSH is regulated by NADPH, which is an essential co-factor necessary to convert GSSG to GSH [21,22]. Several studies have demonstrated that both NADPH and GSH levels are associated with the degree of oxidative stress as well as I/R injury [43,44]. In the present study we found that I/R significantly decreased NADPH levels in the non-pre-conditioned mouse, whereas I/R in the pre-conditioned mouse increased NADPH levels significantly.

In the kidneys, we have reported that IDH1 is a major enzyme that produces NADPH. IDH1 over-expression in cultured kidney epithelial cells reduced oxidative stress and protected cells from oxidative stress-induced cell death, whereas inhibition of IDH1-expression using siRNA against IDH1 mRNA increased susceptibility to oxidative stress-induced cell death [27]. In addition, I/R reduced the activity and expression of IDH1 in kidneys, which in turn decreased NADPH production [27]. Expression and activity of IDH1 differ in various kidney regions; expression of IDH1 is low in the S3 segment of the proximal tubule which is most sensitive to I/R-induced injury [27]. Up-regulation of IDH1 expression in cultured fibroblasts decreases cell susceptibility to oxidative stress, whereas down-regulation of IDH1 expression increases cell susceptibility [45]. Therefore, we compared IDH1 activities in kidneys of pre-conditioned and non-pre-conditioned mice. Our results showed that IDH1 activity after I/R was significantly increased in pre-conditioned mouse kidneys and correlated with NADPH levels, whereas the activity of IDH1 and NADPH was decreased in non-pre-conditioned mouse kidneys. Levels of IDH1 expression positively correlate with kidney cell resistance to I/R-induced oxidative stress. It suggests that increased activity of IDH1 in the pre-conditioned mouse contributes to the protection afforded by ischemic pre-conditioning.

In the present study the decrease of G6PD activity 24 h after ischemia was less than IDH1 activity. Eight days after ischemia (when renal function returned almost normal), G6PD activity dropped to a lower level than 24 h after ischemia (when the decrease of renal function was peaked), whereas IDH1 activity returned to normal ranges 8 days after ischemia. This suggests that IDH1 rather than G6PD is a major contributor in NADPH production. Recently, we reported that G6PD produced 10–30-times less NADPH than IDH1 within the kidney [27] and that IDH1 is highly expressed in the kidney [46].

Our data demonstrate that the increased activity of IDH1 in kidneys of the pre-conditioned mouse is, at least in part, associated with pre-conditioning-induced protection through the serial activation of the IDH1-NADPH-GSH axis.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Chen W, Gabel S, Steenbergen C, Murphy E. A redox-based mechanism for cardioprotection induced by ischemic preconditioning in perfused rat heart. Circ Res 1995;77:424–429.

[2] Soncul H, Oz E, Kalaycioglu S. Role of ischemic preconditioning on ischemia-reperfusion injury of the lung. Chest 1999;115:1672–1677.

[3] Lee HT, Emala CW. Protective effects of renal ischemic preconditioning and adenosine pretreatment: role of A(1) and A(3) receptors. Am J Physiol Renal Physiol 2000;278:380–387.

[4] Joo JD, Kim M, D'Agati VD, Lee HT. Ischemic preconditioning provides both acute and delayed protection against renal ischemia and reperfusion injury in mice. J Am Soc Nephrol 2006;17:3115–3123.

[5] Stagliano NE, Perez-Pinzon MA, Moskowitz MA, Huang PL. Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice. J Cereb Blood Flow Metab 1999;19:757–761.

[6] Sun XC, Li WB, Li QJ, Zhang M, Xian XH, Qi J, et al. Limb ischemic preconditioning induces brain ischemic tolerance via p38 MAPK. Brain Res 2006;1084:165–174.

[7] Park KM, Chen A, Bonventre JV. Prevention of kidney ischemia/reperfusion-induced functional injury and JNK, p38, and MAPK kinase activation by remote ischemic pretreatment. J Biol Chem 2001;276:11870–11876.

[8] Park KM, Byun JY, Kramers C, Kim JI, Huang PL, Bonventre JV. Inducible nitric-oxide synthase is an important contributor to prolonged protective effects of ischemic preconditioning in the mouse kidney. J Biol Chem 2003; 278:27256–27266.

[9] Kim J, Kil IS, Seok YM, Yang ES, Kim DK, Lim DG, et al. Orchiectomy attenuates post-ischemic oxidative stress and ischemia/reperfusion injury in mice. A role for manganese superoxide dismutase. J Biol Chem 2006;281:20349–20356.

[10] Dobashi K, Ghosh B, Orak JK, Singh I, Singh AK. Kidney ischemia-reperfusion: modulation of antioxidant defenses. Mol Cell Biochem 2000;205:1–11.

[11] Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95.

[12] Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. Faseb J 1996;10:709–720.

[13] Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 2001;31:1287–1312.

[14] Land W, Schneeberger H, Schleibner S, Illner WD, Abendroth D, Rutili G, et al. The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. Transplantation 1994;57:211–217.

[15] Lui SL, Chan LY, Zhang XH, Zhu W, Chan TM, Fung PC, Lai KN. Effect of mycophenolate mofetil on nitric oxide production and inducible nitric oxide synthase gene expression during renal ischaemia-reperfusion injury. Nephrol Dial Transplant 2001;16:1577–1582.

[16] Garcia-Criado FJ, Eleno N, Santos-Benito F, Valdunciel JJ, Reverte M, Lozano-Sanchez FS, et al. Protective effect of exogenous nitric oxide on the renal function and inflammatory response in a model of ischemia-reperfusion. Transplantation 1998;66:982–990.

[17] Veech RL, Eggleston LV, Krebs HA. The redox state of free nicotinamide-adenine dinucleotide phosphate in the cytoplasm of rat liver. Biochem J 1969;115:609–619.

[18] Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C, et al. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell 2010;143:802–812.

[19] Beer SM, Taylor ER, Brown SE, Dahm CC, Costa NJ, Runswick MJ, Murphy MP. Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: implications for mitochondrial redox regulation and antioxidant DEFENSE. J Biol Chem 2004;279:47939–47951.

[20] Sztajer H, Gamain B, Aumann KD, Slomianny C, Becker K, Brigelius-Flohe R, Flohe L. The putative glutathione peroxidase gene of Plasmodium falciparum codes for a thioredoxin peroxidase. J Biol Chem 2001;276:7397–7403.

[21] Thon M, Al-Abdallah Q, Hortschansky P, Brakhage AA. The thioredoxin system of the filamentous fungus Aspergillus nidulans: impact on development and oxidative stress response. J Biol Chem 2007;282:27259–27269.

[22] Flamigni F, Marmiroli S, Caldarera CM, Guarnieri C. Involvement of thiol transferase- and thioredoxin-dependent systems in the protection of 'essential' thiol groups of ornithine decarboxylase. Biochem J 1989;259:111–115.

[23] Koh HJ, Lee SM, Son BG, Lee SH, Ryoo ZY, Chang KT, et al. Cytosolic NADP+-dependent isocitrate dehydrogenase plays a key role in lipid metabolism. J Biol Chem 2004;279: 39968–39974.

[24] Jennings GT, Stevenson PM. A study of the control of NADP(+)-dependent isocitrate dehydrogenase activity during gonadotropin-induced development of the rat ovary. Eur J Biochem 1991;198:621–625.

[25] Frederiks WM, Kummerlin IP, Bosch KS, Vreeling-Sindelarova H, Jonker A, Van Noorden CJ. NADPH production by the pentose phosphate pathway in the zona fasciculata of rat adrenal gland. J Histochem Cytochem 2007;55:975–980.

[26] Kloosterhof NK, Bralten LB, Dubbink HJ, French PJ, van den Bent MJ. Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma? Lancet Oncol 2010;12:83–91.

[27] Kim J, Kim KY, Jang HS, Yoshida T, Tsuchiya K, Nitta K, et al. Role of cytosolic NADP+-dependent isocitrate dehydrogenase in ischemia-reperfusion injury in mouse kidney. Am J Physiol Renal Physiol 2009;296:622–633.

[28] Lee SM, Koh HJ, Park DC, Song BJ, Huh TL, Park JW. Cytosolic NADP(+)-dependent isocitrate dehydrogenase status modulates oxidative damage to cells. Free Radic Biol Med 2002;32:1185–1196.

[29] Thadhani R, Pascual M, Bonventre JV. Acute renal failure. N Engl J Med 1996;334:1448–1460.

[30] Thakar CV, Worley S, Arrigain S, Yared JP, Paganini EP. Improved survival in acute kidney injury after cardiac surgery. Am J Kidney Dis 2007;50:703–711.

[31] Kim J, Kim JI, Kwon TH, Park KM. Kidney tubular cell regeneration starts in the deep cortex after ischemia. Korean J Nephrol 2008;27:536–544.

[32] Seok YM, Kim J, Choi KC, Yoon CH, Boo YC, Park Y, Park KM. Wen-pi-tang-Hab-Wu-ling-san attenuates kidney ischemia/reperfusion injury in mice. A role for antioxidant enzymes and heat-shock proteins. J Ethnopharmacol 2007; 112:333–340.

[33] Kim SY, Park JW. Cellular defense against singlet oxygen-induced oxidative damage by cytosolic NADP+-dependent isocitrate dehydrogenase. Free Radic Res 2003;37:309–316.
[34] Stanton RC, Seifter JL. Epidermal growth factor rapidly activates the hexose monophosphate shunt in kidney cells. Am J Physiol 1988;254:267–271.
[35] Zerez CR, Lee SJ, Tanaka KR. Spectrophotometric determination of oxidized and reduced pyridine nucleotides in erythrocytes using a single extraction procedure. Anal Biochem 1987;164:367–373.
[36] Akerboom TP, Sies H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Meth Enzymol 1981;77:373–382.
[37] Jain M, Cui L, Brenner DA, Wang B, Handy DE, Leopold JA, et al. Increased myocardial dysfunction after ischemia-reperfusion in mice lacking glucose-6-phosphate dehydrogenase. Circulation 2004;109:898–903.
[38] Kim J, Seok YM, Jung KJ, Park KM. Reactive oxygen species/oxidative stress contributes to progression of kidney fibrosis following transient ischemic injury in mice. Am J Physiol Renal Physiol 2009;297:F461–F470.
[39] Allen RG, Balin AK. Effects of oxygen on the antioxidant responses of normal and transformed cells. Exp Cell Res 2003;289:307–316.
[40] Balin AK, Pratt L, Allen RG. Effects of ambient oxygen concentration on the growth and antioxidant defenses of human cell cultures established from fetal and postnatal skin. Free Radic Biol Med 2002;32:257–267.
[41] Mandel LJ, Schnellmann RG, Jacobs WR. Intracellular glutathione in the protection from anoxic injury in renal proximal tubules. J Clin Invest 1990;85:316–324.
[42] Paller MS, Patten M. Protective effects of glutathione, glycine, or alanine in an *in vitro* model of renal anoxia. J Am Soc Nephrol 1992;2:1338–1344.
[43] Kim J, Park JW, Park KM. Increased superoxide formation induced by irradiation preconditioning triggers kidney resistance to ischemia-reperfusion injury in mice. Am J Physiol 2009;296:1202–1211.
[44] Vlessis AA, Mela-Riker L. Potential role of mitochondrial calcium metabolism during reperfusion injury. Am J Physiol 1989;256:1196–1206.
[45] Lee SH, Jo SH, Lee SM, Koh HJ, Song H, Park JW, et al. Role of NADP+ -dependent isocitrate dehydrogenase (NADP+ -ICDH) on cellular defence against oxidative injury by gamma-rays. Int J Radiat Biol 2004;80:635–642.
[46] Jo SH, Son MK, Koh HJ, Lee SM, Song IH, Kim YO, et al. Control of mitochondrial redox balance and cellular defense against oxidative damage by mitochondrial NADP+ -dependent isocitrate dehydrogenase. J Biol Chem 2001;276:16168–16176.

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