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Effect of Ischemia–Reperfusion on Renal Expression and Activity of N^G-N^G-Dimethylarginine Dimethylaminohydrolases

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Background: **Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase. It is degraded by the enzyme dimethylarginine dimethylaminohydrolase (DDAH).**

Methods: **Rats (n = 50) underwent to 45 min of renal ischemia followed by 30 min, 1 h, and 3 h of reperfusion. Expression of endothelial nitric oxide synthase, inducible nitric oxide synthase, DDAH-1, DDAH-2, renal DDAH activity, plasma NO_2^-/NO_3^-, and ADMA levels were evaluated.**

Results: **Inducible nitric oxide synthase expression increased, as confirmed by both plasma (11.89 ± 1.02, 15.56 ± 0.93, 11.82 ± 0.86, 35.05 ± 1.28, and 43.89 ± 1.63 nmol/ml in the control, ischemic, 30-min, 1-h, and 3-h groups, respectively) and renal (4.81 ± 0.4, 4.85 ± 1, 9.42 ± 0.7, 15.42 ± 0.85, and 22.03 ± 1.11 nmol/mg protein) formations of NO_2^-/NO_3^-. DDAH-1 expression decreased after reperfusion, whereas DDAH-2 increased after 30 min, returning to basal levels after 3 h. Total DDAH activity was reduced during all times of reperfusion. Both plasma (0.41 ± 0.03, 0.43 ± 0.05, 0.62 ± 0.02, 0.71 ± 0.02, and 0.41 ± 0.01 nmol/ml in the control, ischemic, 30-min, 1-h, and 3-h groups, respectively) and renal (1.51 ± 0.01, 1.5 ± 0.01, 1.53 ± 0.01, 2.52 ± 0.04, and 4.48 ± 0.03 nmol/mg protein in the control, ischemic, 30-min, 1-h, and 3-h groups, respectively) concentrations of ADMA increased.**

Conclusions: **Results suggest that ischemia–reperfusion injury leads to reduced DDAH activity and modification of different DDAH isoform expression, thus leading to increased ADMA levels, which may lead to increased cardiovascular risk.**

RENAL ischemia is a consequence of arterial occlusion, shock, and organ transplantation and is a common cause of renal cell death,[1] delayed graft function,[2,3] renal graft rejection,[4] and acute renal failure (ARF).[5] Despite significant advances in critical care medicine, ARF remains a major clinical problem, and mortality associated with ARF has not decreased substantially in the past 50 yr. The mortality rate of ARF remains between 50% and 70% among patients in intensive care who require dialysis, and ranges between 25% and 100% in postoperative patients with ARF.[6–8] The prognosis is complicated by the fact that reperfusion, although essential for the survival of ischemic renal tissue, causes additional damage, contributing to the renal dysfunction and injury associated with ischemia–reperfusion injury (I-R) of the kidney.[9]

Renal I-R results in cellular injury triggering a complex series of biochemical events, among which nitric oxide release seems to play a key role.[10] Nitric oxide, generated by any of the three isoforms of nitric oxide synthase (NOS), plays an important role in renal function, under both normal and pathophysiologic conditions.[11–14] Three isoforms of NOS have been located in the kidney[12]; the endothelial isoform (eNOS) has been identified in the renal vasculature,[12] and inducible NOS (iNOS) can be induced in the kidney by cytokines, by lipopolysaccharide, and during I-R leading to renal cell injury,[15] whereas neuronal NOS is present throughout the cytoplasm of macula densa and has been involved in the regulation of tubuloglomerular feedback and glomerular capillary pressure.[12,13] To this regard, numerous *in vivo* and *in vitro* investigations have demonstrated how inhibition of the expression or activity of iNOS,[16] or absence of iNOS itself,[17] can ameliorate or prevent renal I-R injury, suggesting that nitric oxide generated by iNOS contributes to renal I-R injury.

Recently, it was recognized that endogenous arginine analogs may play a regulatory role in the arginine–nitric oxide pathway. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of all isoforms of NOS. In asymptomatic patients with hypercholesterolemia, elevated levels of ADMA were found, and ADMA levels were associated with impaired endothelium-dependent vasodilatation and reduced nitrate excretion.[18,19] ADMA levels also increased in elderly patients with peripheral arterial disease and generalized atherosclerosis.[20,21] Most intriguingly, it was shown that ADMA levels were significantly correlated with carotid artery intima-media thickness in stepwise regression analysis, and ADMA increase precedes the occurrence of vascular occlusive disease.[20,22,23] As far as concerns chronic renal disease, Vallance *et al.*[24] reported elevated levels of ADMA in patients with renal failure. Moreover, Nijveldt *et al.*[25] confirmed the role for the kidney in the regulation of plasma levels of ADMA, because it was significantly ex-

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tracted from the arterial supply of the human kidney. Taken together, these observations suggest that plasma ADMA is significantly associated with increased cardiovascular risk.

Asymmetric dimethylarginine is thought to be eliminated from the body by enzymatic degradation by dimethylarginine dimethylaminohydrolase (DDAH). This enzyme has been isolated from rat kidney and is colocalized with the different NOS enzymes, and its reduced activity may account for elevated ADMA concentrations.[26] However, despite several studies that have been performed to elucidate the role of DDAH isoforms in the kidney, we are not aware of previous studies regarding the effects of renal I-R on DDAH isoforms expression, DDAH activity, and ADMA determination in both plasma and tissue.

This study was aimed at elucidating the effects of renal I-R injury on the expression of DDAH isoforms, DDAH activity, and ADMA modifications.

Materials and Methods

Induction of Renal Ischemia and Tissue Preparation

All efforts were made to minimize both the suffering and number of animals used. All experiments were approved and conformed to the guidelines of the Ethical Committee of the University of Catania (Catania, Italy). Rats (n = 50) were subjected to urethane anesthesia (1.2 g/kg, intraperitoneal), and then subjected to 45 min of bilateral renal ischemia by means of occlusion of both renal arteries using vascular clips with a closing force of 0.95 N. Reperfusion was allowed by means of clip removal for 30 min (n = 10), 1 h (n = 10), or 3 h (n = 10). Control rats (n = 10) were sham operated with mobilization of kidney but no clamping of arteries. A separate group of animals (n = 10) were also killed after only 45 min of ischemia. In a second set of experiments, rats were treated with ADMA (3 mg/kg) 3 h before induction of ischemia or sham operation. Rats were killed, heparinized venous blood samples were collected, and both kidneys were removed and frozen at −80°C. All surgical manipulations were performed under normothermic conditions. Unless otherwise specified, kidneys were homogenized in cold phosphate-buffered solution (pH 7.4; 1:10 wt/vol) containing protease inhibitor cocktail (1:1,000; Sigma-Aldrich, Milan, Italy) and 2 mM (final concentration) β-mercaptoethanol. Kidneys were also subjected to protocols described below for immunohistochemical analyses. The number of animals used for biochemical experiments was three to six rats per group, and four rats per group were used for histochemical analysis.

Western Blot Analysis

Whole kidney homogenates were processed for Western blot analysis and protein levels were visualized by immunoblotting as previously described[27] with antibodies against eNOS (Stressgen Biotechnologies Corp., Victoria, British Columbia, Canada), iNOS (Santa Cruz Biotechnology, Santa Cruz, CA), DDAH-1, or DDAH-2 (Oncogene, Cambridge, MA). Briefly, 30 μg protein was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Amersham Inc., Piscataway, NJ) using a semidry transfer apparatus (Bio-Rad, Hercules, CA). The membranes were incubated with 5% milk in 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.05% Tween 20 buffer at 4°C overnight. After washing with 150 mM NaCl and 0.05% Tween 20 buffer, the membranes were incubated with a 1:1,000 dilution of specific antibody overnight with constant shaking. The filters were then washed and subsequently probed with horseradish peroxidase–conjugated donkey anti-rabbit immunoglobulin G (Amersham, Milan, Italy) at a dilution of 1:2,000. Chemiluminescence detection was performed with the Amersham Enhanced Chemiluminescence detection kit according to the manufacturer's instructions. Densitometric analysis was then performed and normalized with relative actin (Santa Cruz Biotechnology).

Immunohistochemical Analysis

Immunostaining for iNOS was performed as previously described[28] in serial sections of rat kidneys immersed in 3% hydrogen peroxide diluted in methanol for 30 min to block endogenous peroxidase activity. The sections were incubated with goat serum (Dakopatts, Milan, Italy; diluted 1:5) for 40 min and then successively with rabbit polyclonal anti-eNOS and iNOS antibodies (Santa Cruz Biotechnology; diluted 1:100) for 2 h. The sections were washed in Tris-buffered saline (0.1 M, pH 7.4) and subsequently incubated with biotinylated goat anti-rabbit immunoglobulin and avidin–biotin–horseradish peroxidase complex according to the manufacturer's instructions (ABC kit; Dakopatts). Specificities of iNOS staining were assessed using appropriate controls and by incubating the tissue sections with nonimmune rabbit serum. The immunoreactivity for iNOS was then analyzed by specific software (Image Pro Plus; Milan, Italy) to give integrated optical densities of different sections.

Histologic Analysis

Tissue samples were fixed in 4% paraformaldehyde; we obtained 5-mm sections and stained them with the periodic acid–Schiff reagent. Twenty tubules or glomeruli in each kidney were randomly selected, and the degree of renal damage was scored using the scoring system for renal injury reported by Solez *et al.* [29] We calculated the mean renal injury score in each animal and then averaged the scores for each group. The sec-

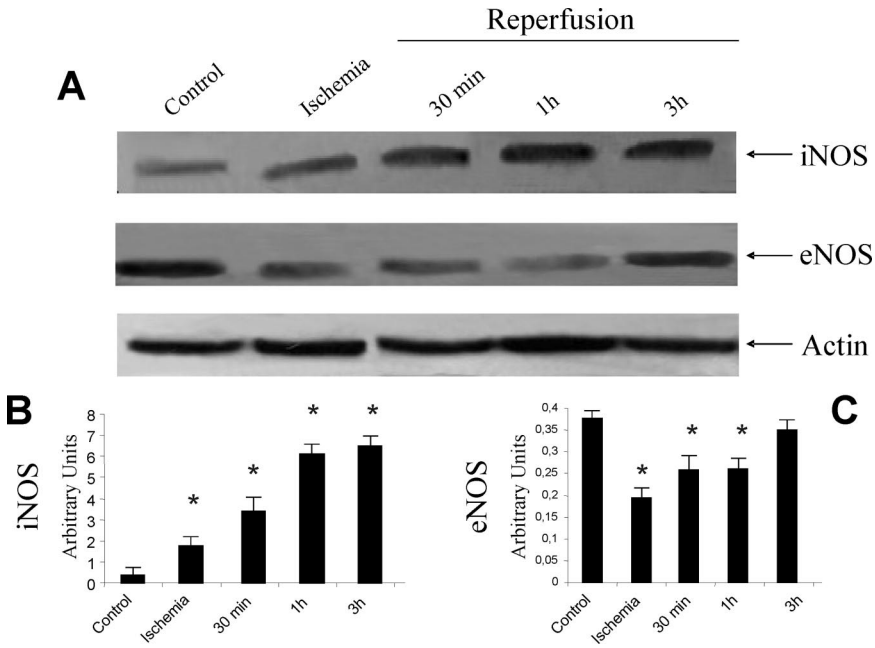

Fig. 1. (*A*) Endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) protein levels in whole kidney homogenate after ischemia–reperfusion were visualized by immunoblotting with antibodies against eNOS and iNOS. (*B* and *C*) Densitometric analysis was performed after normalization with actin. Blots shown are representative of Western blot analysis from four separate experiments (* $P < 0.05$ *vs.* sham-operated animal).

tions were examined by a pathologist in a blinded manner. We examined the tissues for the presence of expansion of Bowman space, interstitial edema, epithelial detachment, and tubular cells casts. Renal morphologic changes were graded on a scale of 0 to 3+: 0, normal; 1+, slight; 2+, moderate; and 3+, severe.

NO_2^-/NO_3^- Quantification

Nitrite, the stable metabolite of nitric oxide, was measured colorimetrically *via* Griess reaction. Aliquots of homogenates were preincubated for 30 min at room temperature with 50 μM nicotinamide adenine dinucleotide phosphate (Sigma-Aldrich, St. Louis, MO) and 24 mU nitrate reductase (Roche Diagnostics Gmbh, Mannheim, Germany), and then the samples were treated with 0.2 U lactate dehydrogenase (Roche) and 0.5 μmol sodium pyruvate for 10 min. The coloration was developed adding Griess reagent (Merck KGaA, Darmstadt, Germany; 1:1, vol/vol). Finally, after 10 min at room temperature, absorbance was recorded by 96-well plate microtiter (Thermo Labsystems Multiskan, Milford, MA) at $\lambda = 540$ nm. Nitrite levels were determined using a standard curve and expressed as nanomoles of NO_2^-/NO_3^- per milligram of protein. Protein content was determined by Lowry assay.

DDAH Enzyme Activity Assay and Determination of ADMA

Tissues were homogenized in 0.1 M phosphate buffer, pH 6.5, containing 2 mM mercapto-ethanol and protease inhibitor cocktail (1:1,000); homogenates were centrifuged at 5,000*g* for 60 min, and supernatants were collected for DDAH enzyme activity assay. DDAH enzyme activity was assayed by determining L-citrulline formation in a 96-well microtiter plate according to Knipp and Vasak.[30] One unit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol L-citrulline/min at 37°C. Plasma and tissue ADMA concentration was determined by using a commercially available enzyme-linked immunosorbent assay kit (DLD Diagnostika GmbH, Hamburg, Germany) according to the manufacturer's instructions.

Assessment of Renal Function

Plasma creatinine and urea nitrogen was detected by a kinetic enzymatic procedure (Urea Nitrogen; Abbott Diagnostics, Milan, Italy) by using an autoanalyzer (ARCHITECT CI 8200; Abbott Diagnostics). The limits of detection for creatinine and blood urea nitrogen are 0.05 and 0.7 mg/dl, respectively.

Statistical Analysis

One-way analysis of variance followed by Bonferroni posttest (GraphPad Software, Inc., San Diego, CA) was performed to estimate significant differences among groups. The correlation between renal injury score and ADMA was assessed by linear regression analysis. Data were reported as mean ± SD, and differences between groups were considered to be significant at $P < 0.05$.

Results

eNOS and iNOS Expression after I-R

Western blot analysis demonstrated a time-dependent increase of iNOS expression peaking at 1 h of reperfusion (figs. 1A and B). By contrast, eNOS expression showed a significant decrease after ischemia alone or I-R and was restored only after 3 h of reperfusion (figs. 1A and C). To confirm Western blot results and to evaluate

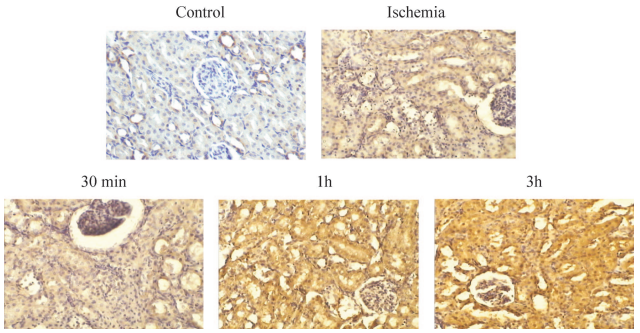

Fig. 2. Light micrographs illustrating immunostaining for inducible nitric oxide synthase along different segments of the nephron. Each micrograph is representative of four separate experiments. Magnification ×180.

iNOS expression along different nephron segments, we performed immunohistochemistry followed by an integrated optical density analysis (fig. 2). These results confirmed the results obtained by Western blot analysis showing a marked increase along all different segments of the nephron of iNOS expression following 1 h of reperfusion.

Plasma and Tissue Determination of NO_2^-/NO_3^-

To evaluate whether variations in protein expression were also followed by modification of the enzymatic activity,we determined NOS activity as measured by NO_2^-/NO_3^- formation (figs. 3A and B). This set of experiments was consistent with iNOS expression showing a significant increase of NO_2^-/NO_3^- formation in both kidney and plasma after I-R. Consistently with Western blot results, plasma NO_2^-/NO_3^- formation was significantly increased ($P < 0.01$) 1 h after reperfusion and further increased after 3 h (11.89 ± 1.02, 15.56 ± 0.93, 11.82 ± 0.86, 35.05 ± 1.28, and 43.89 ± 1.63 nmol/ml in the control, ischemic, 30-min, 1-h, and 3-h groups, respectively). By contrast, tissue formation of NO_2^-/NO_3^- was significantly increased ($P < 0.01$) after 30 min of reperfusion (4.81 ± 0.4, 4.85 ± 1, 9.42 ± 0.7, 15.42 ± 0.85, and 22.03 ± 1.11 nmol/mg protein in the control, ischemic, 30-min, 1-h, and 3-h groups, respectively).

Histologic Analysis

None of the animals died when the renal arteries were clamped for 45 min. Figure 4A shows the renal histology stained with periodic acid–Schiff reagent. In the vehicle group, remarkable time-dependent damage, particularly in the tubuli, was observed. Renal damage included detachment of epithelial cells of the tubuli, interstitial edema, and many tubular cell casts. Bowman space was also remarkably expanded. The renal injury scores of the five groups are shown in figure 4B. The I-R procedure resulted in a significantly ($P < 0.05$) time-dependent increase in the injury scores (control, 0.6 ± 0.1; ischemia, 1.74 ± 0.3; reperfusion 30 min, 1.82 ± 0.5; reperfusion 1 h, 3.5 ± 0.6; reperfusion 3 h, 5.37 ± 0.2).

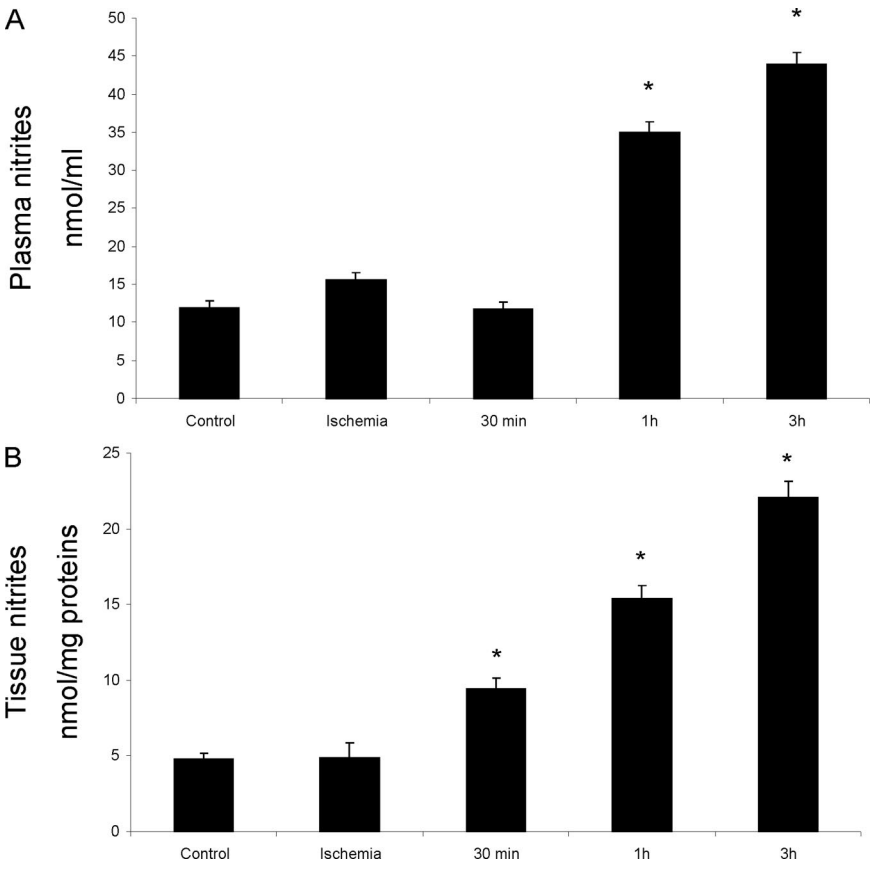

Fig. 3. NO_2/NO_3 levels in plasma (*A*) and tissue (*B*) after renal ischemia–reperfusion. Rats were subjected to 45 min of bilateral renal ischemia followed by 30 min, 1 h, or 3 h of reperfusion. NO_2/NO_3 formation was determined after the Griess reaction. Values are the mean ± SD of four experiments in duplicate (* $P < 0.01$ *vs.* sham-operated animal).

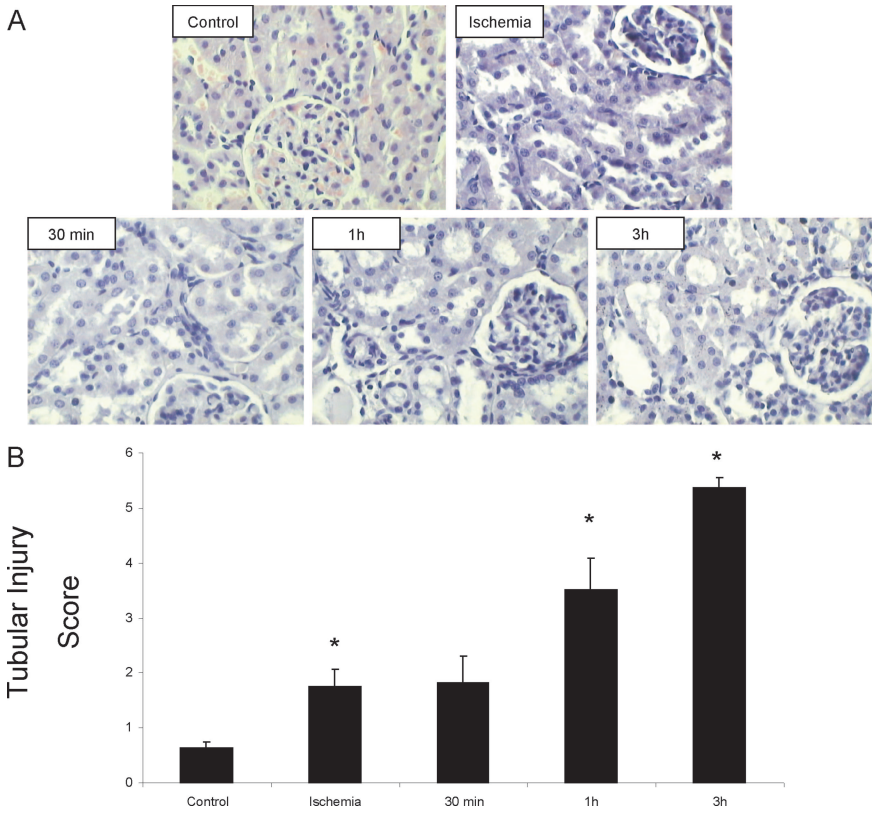

Fig. 4. (*A*) Renal histology showing time-dependent damage that was more evident in the tubuli. (*B*) Scores showing a time-dependent increase in tubular injury (* $P < 0.01$ *vs.* sham-operated animal).

DDAH Isoform Expression after Ischemia–Reperfusion

We further evaluated DDAH isoforms expression during I-R to evaluate how this enzyme counteract the increase of NO_2^-/NO_3^- formation and iNOS expression. This set of experiments showed a significant reduction of DDAH-1 isoform expression after ischemia which was restored only after 3 h of reperfusion (figs. 5A and B). By contrast, DDAH-2 expression showed a marked increase after the ischemia period and returned at basal levels after 1 h of reperfusion (figs. 5A and C).

DDAH Activity, ADMA Determination, and Assessment of Renal Function

To evaluate whether variations in DDAH proteins expression were also followed by modification of the en-

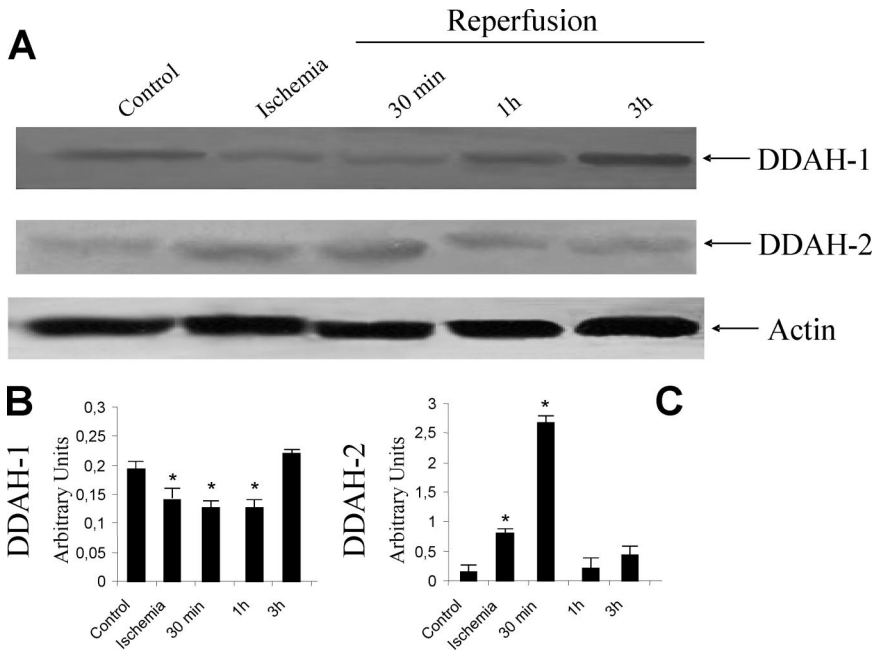

Fig. 5. (*A*) Dimethylarginine dimethylaminohydrolase-1 (DDAH-1) and dimethylarginine dimethylaminohydrolase-2 (DDAH-2) protein levels in whole kidney homogenate after ischemia–reperfusion were visualized by immunoblotting with antibodies against DDAH-1 and DDAH-2. (*B* and *C*) Densitometric analysis was performed after normalization with actin. Blots shown are representative of Western blot analysis from four separate experiments (* $P < 0.05$ *vs.* sham-operated animal).

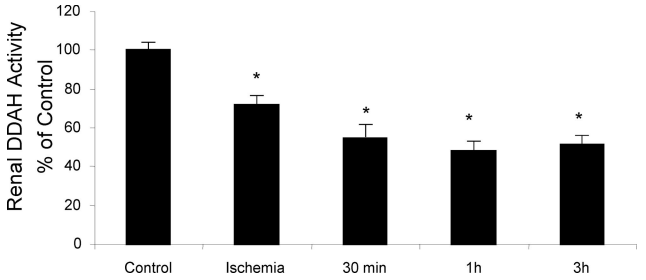

Fig. 6. Renal dimethylarginine dimethylaminohydrolase (DDAH) activity. Homogenates were centrifuged at 5,000*g* for 60 min, and supernatants were used to determine DDAH enzyme activity, which was assayed by determining L-citrulline formation in a 96-well microtiter plate. Values are the mean ± SD of four experiments in duplicate (* *P* < 0.05 *vs.* sham-operated animal).

zymatic activity, we determined total renal DDAH activity. Our results show a significant decrease of DDAH activity after ischemia and reached the lowest value after 1 h of reperfusion (fig. 6). Interestingly, DDAH activity was not restored after 3 h of reperfusion, despite the expression of DDAH-1 and DDAH-2 isoforms being unmodified and increased, respectively, when compared with control.

Furthermore, consistently with reduced DDAH activity, we showed a significant increase of ADMA after 30 min of reperfusion in plasma (0.41 ± 0.03, 0.43 ± 0.05, 0.62 ± 0.02, 0.71 ± 0.02, and 0.41 ± 0.01 nmol/ml in the control, ischemic, 30-min, 1-h, and 3-h groups, respectively) and after 1 h in kidney (1.51 ± 0.01, 1.5 ± 0.01, 1.53 ± 0.01, 2.52 ± 0.04, and 4.48 ± 0.03 nmol/mg protein in the control, ischemic, 30-min, 1-h, and 3-h groups, respectively; figs. 7A and B). No significant correlation was observed between ADMA levels and the grade of tubular injury ($r = 0.09$, $P = 0.87$).

Finally, we tested the effects of exogenous ADMA on renal function after I-R. In this set of experiments, no significant differences were observed for plasma creatinine and blood urea nitrogen after ADMA administration ($P > 0.05$ for all groups) during any time of reperfusion (fig. 8).

Discussion

There is strong evidence that the increase of plasma ADMA plays an important role in the pathophysiology of cardiovascular risk in various renal injury models.[31–33] We demonstrated here that DDAH undergoes modifications after I-R injury, probably in an attempt to overwhelm the increased nitric oxide production. This conclusion is supported by our results showing that DDAH isoform expression and activity undergo time-dependent modifications after I-R. Consistently, ADMA levels, as a result of reduced DDAH activity, were increased in both kidney and plasma. However, the pattern of ADMA levels in these compartments was temporally dissociated, thus

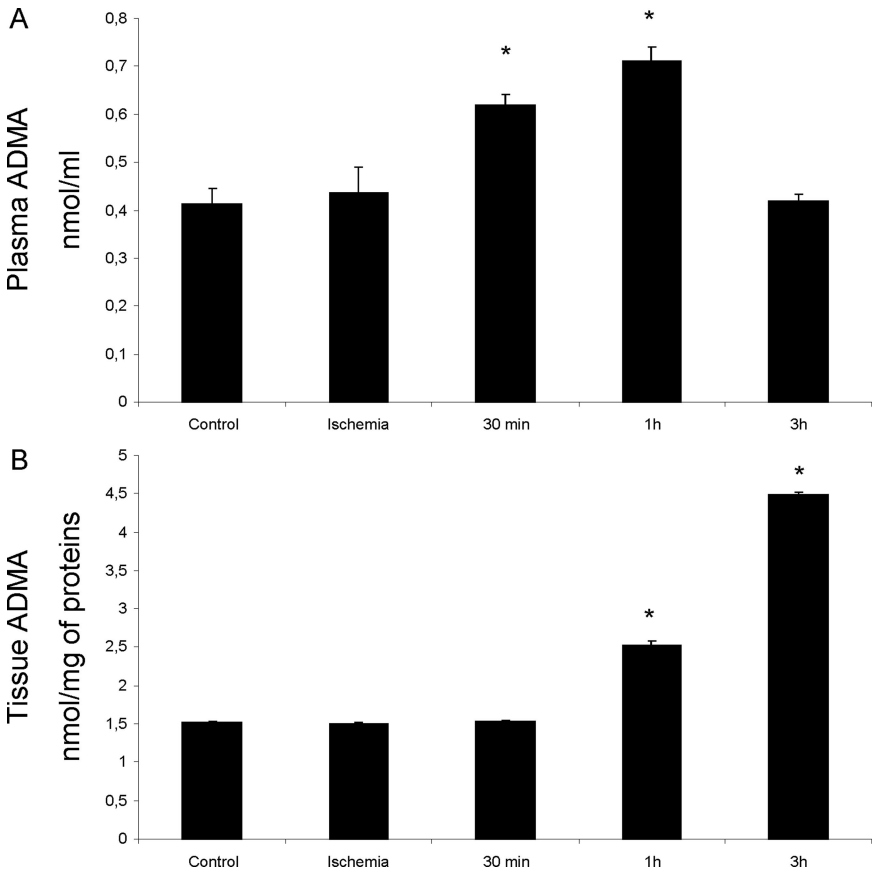

Fig. 7. Plasma (nmol/l; *A*) and renal (nmol/mg protein; *B*) asymmetric dimethylarginine (ADMA) concentrations after ischemia–reperfusion. Values are the mean ± SD of four experiments in duplicate (* *P* < 0.05 *vs.* sham-operated animal).

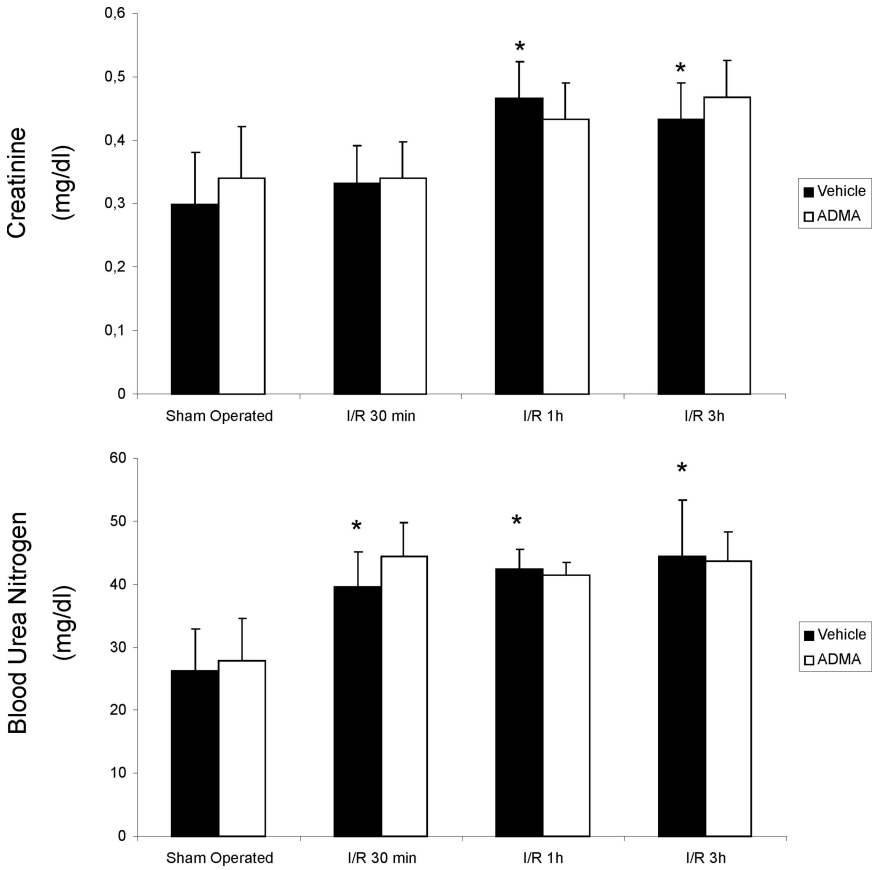

Fig. 8. Plasma creatinine and blood urea nitrogen in sham and ischemia–reperfusion (I/R) rats treated and untreated with asymmetric dimethylarginine (ADMA, 3 mg/kg, 3 h before ischemia induction or sham operation) (* $P < 0.05$ *vs.* sham-operated animal).

suggesting the possibility of extrarenal sources of ADMA. Taken together, our results provide an interesting insight into the renal DDAH enzyme in an *in vivo* experimental model of I-R and may provide the basis for further understanding of the pathophysiologic processes leading to increased cardiovascular risk for renal transplant patients.

To this regard, it has been shown that cardiovascular mortality is elevated in transplant patients as compared with the general population.[34] Importantly, traditional cardiovascular risk factors derived from different epidemiologic studies fall short of explaining cardiovascular morbidity in these patients. Consequently, this has led to investigations into novel, putative risk factors that may explain this discrepancy. One major pathophysiologic phenomenon observed even in transplant patients without evidence of overt cardiovascular disease is dysfunction of the endothelium.[35] Our results are consistent with this hypothesis because we showed that I-R results in reduced DDAH activity, which leads to ADMA accumulation in both kidney and plasma. In particular, plasma ADMA accumulation may lead to nonselective inhibition of NOS isoforms in various organs, thus leading to systemic adverse effects such as oxidative stress, endothelial and cardiac dysfunction, compromised flow-mediated vasodilatation, vascular remodeling, and hypertension (fig. 9).

Previous work of Kielstein *et al.*[36,37] also support this hypothesis; in fact, they suggested that ADMA is higher in dialysis patients with concomitant cardiovascular disease than in dialysis patients without cardiovascular complications, suggesting that either elevated ADMA

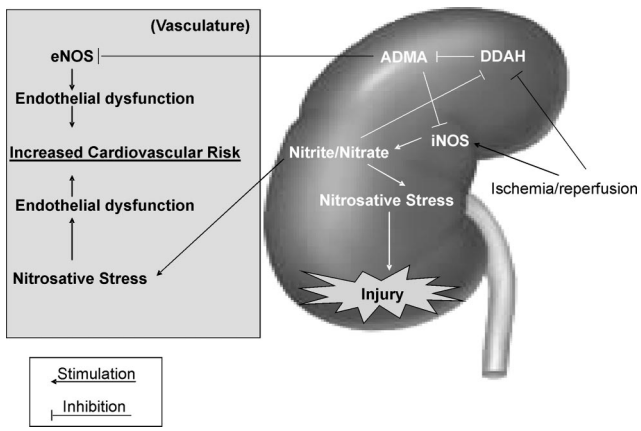

Fig. 9. Schematic overview of biochemical pathway, which in turn may lead to increased cardiovascular risk after renal ischemia–reperfusion. On one hand, ischemia–reperfusion leads to increased inducible nitric oxide synthase (iNOS) expression and activity, causing nitrosative stress and thus renal injury. Furthermore, nitrites are released into plasma, thus leading to endothelial dysfunction. On the other hand, increased nitrite formation leads to reduced dimethylarginine dimethylaminohydrolase (DDAH) activity and expression, causing asymmetric dimethylarginine (ADMA) accumulation in both kidney and plasma, where it may cause inhibition of endothelial nitric oxide synthase (eNOS) and thus endothelial dysfunction. This pathophysiologic process, together with others, such as inflammatory cytokines release, may concur with the increased cardiovascular risk.

caused cardiovascular complications in this subgroup, or cardiovascular disease and renal failure led to elevated ADMA concentration *via* independent mechanisms. Furthermore, the authors showed that patients with a relatively higher plasma concentration of ADMA had a significantly shorter survival than those with a relatively lower plasma concentration. In particular, their results revealed that a 2-μM increase in plasma ADMA is associated with a 37% increase in risk for fatal and nonfatal cardiovascular events. According to our results, it is reasonable to hypothesize that ADMA elevation is partially due to reduced DDAH activity and DDAH-1 expression into the kidney. Furthermore, I-R impaired kidney is not able to eliminate ADMA, which therefore further accumulates into the systemic circulation. Recently, it was shown, by significant extraction of ADMA from the arterial supply of the human kidney, that the kidney is an important contributor in the regulation of plasma levels of dimethylarginines.[25] These authors also showed that the elimination of ADMA by the rat kidney could not be explained by urinary excretion, because urinary concentration of unchanged ADMA was negligible. This finding points to a high metabolic turnover of ADMA in the kidney, which is fully responsible for the observed net renal uptake of ADMA. Although we cannot elucidate the molecular mechanism leading to reduced DDAH activity, in the current study strong evidence was obtained for increased ADMA concentration in both plasma and kidney after I-R (fig. 7). Interestingly, we observed an apparent discrepancy between DDAH isoform expression and activity. This may be related to posttranscriptional modification of DDAH by increased nitric oxide production. In this regard, it is well known that increased nitric oxide levels may lead to reduced DDAH activity by nitrosylation at specific sites.[38]

A question that remains to be resolved, then, is what the real clinical significance of reduced DDAH activity is, because it seems that increased ADMA levels are not able to counteract the increased nitric oxide production and may therefore increase the cardiovascular risk by accumulating into plasma at least in the early phase of reperfusion. Finally, our results showed that exogenous ADMA administration did not significantly change renal function when compared with untreated animals. These results apparently disagree with the notion that NOS inhibition should results in a significant improvement of renal function; however, they may be partially explained by the fact that ADMA does not discriminate between eNOS (exerting renal-protective effects) and iNOS (exerting detrimental renal effects) isoforms. Furthermore, DDAH isoforms are differently colocalized with NOS isoforms,[26] and therefore, exogenous ADMA administration may not necessarily reflect the physiologic modification of the complex DDAH–NOS system.

In conclusion, our study provides evidence for reduced DDAH activity and changes in isoform pattern of expression after renal I-R, thus opening a new scenario in the understanding of pathophysiologic processes of I-R that may be a relevant target for future strategies in the treatment of transplant patients.

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