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Tyrosol Attenuates Ischemia–Reperfusion-Induced Kidney Injury via Inhibition of Inducible Nitric Oxide Synthase

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ABSTRACT: Tyrosol is a natural phenolic antioxidant compound. Oxidative stress represents one of the important mechanisms underlying ischemia–reperfusion-induced kidney injury. The aim of this study was to investigate the effect of tyrosol against ischemia–reperfusion-induced acute kidney injury. The left kidney of Sprague–Dawley rats was subjected to 45 min of ischemia followed by reperfusion for 6 h. Ischemia–reperfusion caused an increase in peroxynitrite formation and lipid peroxidation. The level of nitric oxide (NO) metabolites and the mRNA of inducible nitric oxide synthase (iNOS) were elevated in ischemia-reperfused kidneys. Administration of tyrosol (100 mg/kg body weight) to rats prior to the induction of ischemia significantly reduced peroxynitrite formation, lipid peroxidation, and the level of NO metabolites. Tyrosol administration also attenuated ischemia–reperfusion-induced NF-κB activation and iNOS expression. Such a treatment improved kidney function. Results suggest that tyrosol may have a protective effect against acute kidney injury through inhibition of iNOS-mediated oxidative stress.

KEYWORDS: *kidney, tyrosol, ischemia–reperfusion, nitric oxide, oxidative stress*

INTRODUCTION

Epidemiologic studies have indicated that moderate consumption of olive oil, one of the major components in the Mediterranean diet, is associated with the reduced risk of morbidity and mortality of diseases such as cancer, cardiovascular diseases, and kidney diseases.[1−4] The health benefits of olive oil are attributed to monounsaturated fatty acids and minor components such as phenolic compounds.[5] Growing evidence indicates that the phenolic compounds in olive oil have antioxidant effects.[6,7] Among the phenolic compounds found in olive oil, tyrosol (2-(4-hydroxyphenyl)-ethanol) (Figure 1) is one of the major natural phenolic compounds present at high concentrations in olive oil[8,9] with antioxidant potential. It has been reported that addition of tyrosol to the culture medium reduces reactive oxygen species (ROS) generation in phorbol 12-myristate 13-acetate (PMA)-activated rat peritoneal leukocytes,[10] in human endothelial cells,[11] and in oxidized-LDL treated Caco-2 intestinal mucosa cells.[12] Moreover, it has been shown that the antioxidant property of tyrosol may also contribute to its neuroprotective effect of tyrosol against oxidative stress-induced injuries.[13]

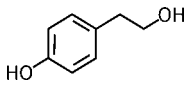

Figure 1. Chemical structure of tyrosol.

Kidney ischemia–reperfusion injury is a clinical complication frequently experienced by patients in the perioperative period.[14] Various clinical–surgical procedures such as renal angioplasty, bypass procedure, and clamping of the renal pedicle or the aorta above the renal arteries lead to a temporary interruption or reduction of blood supply to the kidney tissue.[15,16] The reinstitution of blood to the ischemic area results in ischemia–reperfusion injury,[17] which is associated with prolonged hospitalization and increased morbidity and mortality.[18,19] Studies in patients and in experimental animal models suggest that oxidative stress resulting from the production of ROS plays a key role in ischemia–reperfusion injury.[20−23] Among ROS, superoxide anion can rapidly react with nitric oxide (NO) to form another potent free radical peroxynitrite ($ONOO^-$) that, in turn, causes extensive protein tyrosine nitration.[24,25] NO is an endogenous signal transduction gas molecule and plays an important role in kidney ischemia–reperfusion injury.[26] The NO is synthesized by nitric oxide synthase (NOS), which is found to have three isoforms of NOS, namely, inducible (iNOS), endothelial (eNOS), and neuronal (nNOS), in the kidney. The activation of iNOS often occurs in the kidney under pathological conditions. Excessive production of NO from iNOS exerts detrimental effects to the tissue.[27,28] We have observed that ischemia–reperfusion leads to increased iNOS-mediated NO production and peroxynitrite formation in rat kidney.[23] The expression of the iNOS gene is mainly regulated by a transcription factor, namely, nuclear factor-kappa B (NF-κB). Inhibition of iNOS expression has been shown to attenuate oxidative stress and hence alleviate ischemia–reperfusion injury.[29] Furthermore, inactivation of NF-κB by NF-κB decoy oligodeoxynucleotides treatment prevents ischemia–reperfusion-induced iNOS expression in the kidney.[30]

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Although dialysis is a treatment of choice for acute ischemic kidney failure, it is only a temporary replacement for the lost kidney function.[31,32] The identification of novel therapeutic interventions against kidney ischemia−reperfusion injury has become a subject of intense research interest. Inhibition of iNOS-mediated NO production may antagonize oxidative stress induced by ischemia−reperfusion and hence protect against renal injury. Dietary antioxidants are viewed as a promising therapeutic strategy to combat oxidative stress. As one of the main compounds present in the Mediterranean diet, tyrosol has been reported to have antioxidant activity.[33−35] The ready absorption of tyrosol leads to its effective bioavailability in humans.[36] It has been reported that, once absorbed, tyrosol concentrates mainly in the kidney.[37] However, it remains unclear whether tyrosol can prevent or alleviate ischemia−reperfusion-induced injury in the kidney. Therefore, in the present study, we aimed to investigate the protective effect of tyrosol on ischemia−reperfusion-induced injury in the rat kidney and to identify the underlying mechanisms.

MATERIALS AND METHODS

Renal Ischemia−Reperfusion. Male Sprague−Dawley rats weighing 250−300 g were anesthetized with an intraperitoneal (ip) injection of pentobarbital sodium (50 mg/kg). The rats were kept on a heat pad, and the rectal temperature was maintained at 37 °C throughout the experimental procedure. Kidney ischemia was induced by clamping the left renal vascular pedicle for 45 min with a nontraumatic vascular clamp.[23,38] At the end of ischemia period, the rats were subjected to 6 h of reperfusion by removal of the clamp, and right nephrectomy was performed.[23] A sham-operated group of rats was subjected to the same surgical procedure without inducing ischemia−reperfusion and were sacrificed at corresponding time points. Results obtained from this group were used as controls. In the tyrosol treatment experiment, rats were administered tyrosol [2-(4-hydroxyphenyl)ethanol] (Sigma-Aldrich Chemical Co.) via ip injection 30 min prior to the induction of ischemia followed by reperfusion. A blood sample was drawn from the abdominal aorta before rats were sacrificed. Plasma was separated by centrifugation of blood at 3000*g* for 20 min at 4 °C. Plasma creatinine levels were determined as an index for kidney function by using a Genzyme Diagnostics Creatinine Kit. Kidneys were collected and bisected in ice-cold potassium phosphate buffer. Results obtained from a pilot study indicated that injection of tryosol at 30 mg/kg did not reduce plasma creatinine levels in rats subjected to kidney ischemia−reperfusion. However, injection of tryosol at 50 and 100 mg/kg effectively reduced plasma creatinine levels and improved kidney function. Tryosol at 100 mg/kg displayed more significant effects on renal function than that at 50 mg/kg. Therefore, the 100 mg/kg dosage of tyrosol was chosen in the present study. As this study was conducted in an acute kidney injury model, tyrosol was administered via ip injection to rapidly achieve a peak level of tyrosol in the target organ prior to the induction of renal injury. All of the procedures were performed in accordance with the *Guide to the Care and Use of Experimental Animals*, published by the Canadian Council on Animal Care and approved by the University of Manitoba Protocol Management and Review Committee. All of the chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise indicated.

Determination of Lipid Peroxidation. The degree of lipid peroxidation in the kidney was determined by measurement of malondiadehyde (MDA) levels with thiobarbituric acid reactive substances (TBARS) assay as described previously.[23] A portion of the kidney including both cortex and medulla was homogenized in 0.1 M KCl solution containing 3 mM EDTA, followed by centrifugation at 600*g* for 15 min at 4 °C. An aliquot of supernatant was added to the reaction mixture containing 8% SDS, 20% acetic acid, 0.8% thiobarbituric acid, and water. The reaction mixture was incubated at 95 °C for 1 h. The amount of MDA formed in the reaction mixture was measured by using a spectrophotometer at the absorbance of 532 nm. MDA was used as the external standard, and results were expressed as a percentage of sham-operated group. The amount of MDA levels correlates to the degree of lipid peroxidation produced in the kidney tissues.

Measurement of Nitric Oxide Metabolites in the Kidney. As stable end products of NO metabolites, nitrite and nitrate were measured to assess the NO levels in the kidney. The amount of nitrite and nitrate was determined by Griess reaction assay based on azo coupling reaction as described previously.[39,40] In brief, after the rat was euthanized, a portion of the kidney tissue was immediately placed in a sterile microcentrifuge tube followed by a rapid freezing in liquid nitrogen until assay. Prior to assay, the kidney sample was thawed on ice followed by rapid homogenization in a buffer (pH 7.4) containing 20 mM Tris and 2 mM EDTA and then centrifugation at 600*g* for 10 min at 4 °C. The supernatant was collected and deproteinized with 0.3 N NaOH and 5% $ZnSO_4$. An aliquot of the deproteinized supernatant was incubated with nitrate reductase to convert the nitrate to nitrite. The Griess reaction was initiated by the addition of a reagent containing 12.5 mM sulfanilamide in 6 N HCl and 12.5 mM *N*-(1-naphthyl)ethylenediamine. The absorbency of the reaction mixture was read at 520 nm. Sodium nitrite at different concentrations was used as standards. The concentration of nitrite−nitrate in the kidney tissue was expressed as a percentage of the sham-operated group.

Western Immunoblotting Analysis. iNOS levels in kidney tissue were determined by a Western immunoblotting analysis. In brief, kidney proteins (100 μg) were separated by electrophoresis on an 8% SDS−polyacrylamide gel. The proteins in the gel were transferred to a nitrocellulose membrane. The membrane was probed with rabbit polyclonal anti-iNOS antibodies (1:2000; Calbiochem, Ontario, Canada). For the determination of renal nitrotyrosine protein levels, tissue proteins (40 μg) were separated by electrophoresis on a 15% SDS−polyacrylamide gel, which was followed by electrophoretic transfer of proteins from the gel to a nitrocellulose membrane. The membrane was probed with mouse monoclonal antinitrotyrosine (1:1000, Cayman, Ann Arbor, MI, USA). The bands were visualized by using enhanced chemiluminescence (ECL) reagents and exposed to Kodak BioMax Light film. Film was analyzed by using Bio-Rad Quantity-One image analysis software (version 4.2.1). The same nitrocellulose membrane was probed with mouse monoclonal anti-β-actin antibodies (Cell Signaling Technology, Inc.) to confirm equal loading of proteins of individual samples.

Measurement of iNOS mRNA Expression. Total RNAs were isolated from rat kidney tissues with TriZol reagent (Invitrogen). iNOS mRNA was determined by a real-time PCR analysis using the iQ5 real time PCR detection system (Bio-Rad).[38] In brief, 2 μg of total RNA was reverse transcribed to cDNA in a total volume of 20 μL with the reverse transcription reagent mixer. The reaction mixture of real-time PCR contains 0.4 μM 5′ and 3′ primers, 2 μL of cDNA products, and iQ-SYBR green supermix reagent (Bio-Rad) in a total volume of 25 μL. Crossing threshold values were normalized to β-actin. The nucleotide sequences of primers (Invitrogen) used for rat iNOS (178 bp) were (forward) 5′-GGAAGAAATGCAGGAGATGG-3′ and (reverse) 5′-GCACATCGCCACAAACATAG-3′, and those used for rat β-actin were (forward) 5′-GTCGTACCACTGGCATTGTG-3′ and (reverse) 5′-TCTCAGCTGTGGTGGTGAAG-3′. All of the primers were purchased from Invitrogen. The result of iNOS mRNA expression was expressed as percentage change relative to the sham-operated group.

Electrophoretic Mobility Shift Assay (EMSA). Nuclear proteins were isolated from the kidney tissue, and the NF-κB/DNA binding activity was determined by EMSA.[40] Briefly, 10 μg of nuclear protein was incubated with a reaction buffer for 15 min at room temperature, followed by the incubation with excess ^{32}P-end-labled oligonucleotides containing the NF-κB consensus sequence (Promega, Madison, WI, USA). The sequence of the oligonucleotide probe was 5′-AGTTGA-GGGGACTTCCCAGGC-3′. The radioactive mixture was separated in a 6% nondenaturing polyacrylamide gel and dried on a piece of filter paper followed by autoradiography. The cold competition experiment was performed by adding a 100-fold excess of unlabeled NF-κB probe

prior to the addition of the ^{32}P-end-labeled oligonucleotide in the reaction mixture to confirm that the binding of ^{32}P-end-labled oligonucleotides to NF-κB was sequence specific.

Histological Staining. A portion of the kidney was immersion-fixed in 10% neutral-buffered formalin overnight followed by embedding in paraffin. Sequential 5 μm paraffin-embedded cross sections were prepared and stained with hematoxylin and eosin (H&E) to examine histological changes in the kidney.[23] Images were captured by using Olympus BX43 microscope with an Olympus Q-Color3 digital camera. The slides were analyzed at ×200 magnification using Image-Pro Plus software (version 7.0, Media Cybernetics, Bethesda, MD, USA).

Statistical Analysis. Results were analyzed by using one-way ANOVA followed by Newman−Keuls test. Data were presented as the mean ± SEM. The level of statistical significance was determined when a P value was <0.05.

RESULTS

Effect of Tyrosol on the Function and Morphological Changes in Kidneys Subjected to Ischemia−Reperfusion. As an indicator of kidney dysfunction, plasma creatinine levels were measured. The level of creatinine in rats subjected to 45 min of ischemia followed by 6 h of reperfusion was significantly higher than that in the sham-operated rats (Table 1), indicating impaired kidney function upon ischemia−reperfusion. Kidney tissue morphology was examined by using H&E staining. Tubular necrosis and interstitial congestion were observed in the kidney subjected to ischemia−reperfusion (Figure 2). Administration of tyrosol (100 mg/kg, ip injection) prior to the induction of ischemia caused a decrease in plasma creatinine levels in rats subjected to ischemia−reperfusion (Table 1). In accordance with the improvement of kidney function, tyrosol treatment also protected the structural integrity of the kidney, which was characterized by reduced tubular cell necrosis and lessened interstitial congestion (Figure 2). Administration of tyrosol also preserved the gross appearance of kidney subjected to ischemia−reperfusion (Figure 2).

Table 1. Plasma Creatinine Levels of the Groups

group	n	creatinine (mg/dL)
sham	10	0.42 ± 0.04
I/R	10	1.93 ± 0.13[a]
I/R+Tyr	10	1.51 ± 0.13[a,b]

[a] P < 0.05 vs sham-operated group. [b] P < 0.05 vs ischemia−reperfusion.

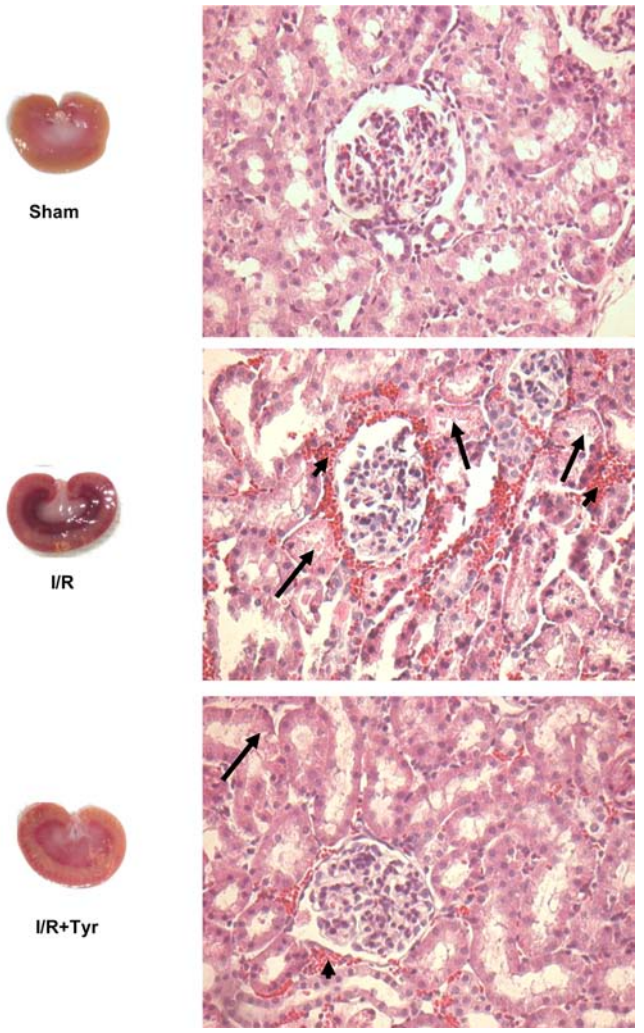

Figure 2. Effect of tyrosol on histological change of renal ischemia−reperfusion. The left kidney was subjected to sham operation (Sham), 45 min of ischemia followed by 6 h of reperfusion (I/R), or 45 min of ischemia followed by 6 h of reperfusion with tyrosol administration (100 mg/kg, intraperitoneal injection) 30 min prior to the induction of ischemia (I/R+Tyr). The gross appearance of a midtransverse plane of kidney was examined. Renal vascular integrity and cellular morphology were examined by hematoxylin and eosin (H&E) staining. A kidney section obtained from the ischemia−reperfusion group showed tubular necrosis and interstitial congestion, compared with the sham-operated group. Long arrows point to the areas with tubular necrosis, and short arrowheads point to the areas with interstitial congestion.

Effect of Tyrosol on Ischemia−Reperfusion-Induced Oxidative Stress in the Kidney. The degree of lipid peroxidation in the kidney was examined by measuring the level of MDA, a stable byproduct of lipid oxidation that is often used as an indicator of tissue oxidative stress. Ischemia−reperfusion resulted in a 2-fold increase in the MDA level in kidneys as compared to that in the sham-operated group, indicating that lipid peroxidation was increased (Figure 3). Administration of tyrosol significantly reduced the degree of lipid peroxidation in the kidney subjected to ischemia−reperfusion (Figure 3). To determine whether there was an increase in peroxynitrite formation in the kidneys subjected to ischemia−reperfusion, Western immunoblotting analysis was carried out to detect nitrotyrosine, a biomarker for peroxynitrite. A significant increase in nitrotyrosine protein adduct was found in the kidneys subjected to ischemia−reperfusion (Figure 4), indicating an increase in peroxynitrite formation as compared to the sham-operated group. Administration of tyrosol significantly reduced the levels of nitrotyrosine protein in the kidneys subjected to ischemia−reperfusion (Figure 4). These results suggested that administration of tyrosol to rats could alleviate ischemia−reperfusion-induced oxidative stress in the kidney.

Effect of Tyrosol on iNOS Expression and NF-κB Activation in the Kidney. Induction of iNOS expression can lead to excessive NO production in the tissue. The NO is able to interact rapidly with superoxide anion to form peroxynitrite, a potent oxidant causing oxidative stress. The effect of ischemia−reperfusion on iNOS expression in the kidney was examined. Ischemia−reperfusion caused a significant increase in iNOS mRNA (Figure 5A) and protein (Figure 5B) levels in the kidney tissue. In accordance, ischemia−reperfusion caused a

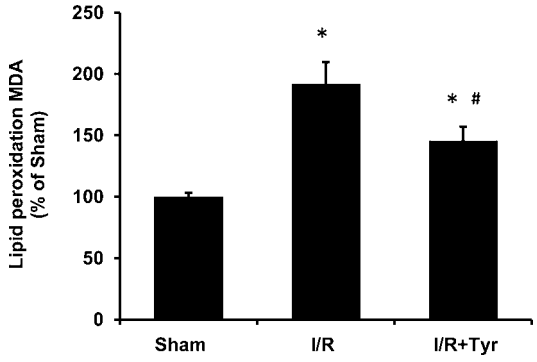

Figure 3. Measurement of lipid peroxidation in the kidney tissue. The left kidney was subjected to sham operation (Sham), 45 min of ischemia followed by 6 h of reperfusion (I/R), or 45 min of ischemia followed by 6 h of reperfusion with tyrosol administration (100 mg/kg, intraperitoneal injection) 30 min prior to the induction of ischemia (I/R+Tyr). Kidney lipid peroxides were determined by measuring the amount of MDA. Results are expressed as the mean ± SEM (n = 10). ∗, $P < 0.05$ when compared with the value obtained from the sham-operated group; #, $P < 0.05$ when compared with the value obtained from the ischemia−reperfusion group.

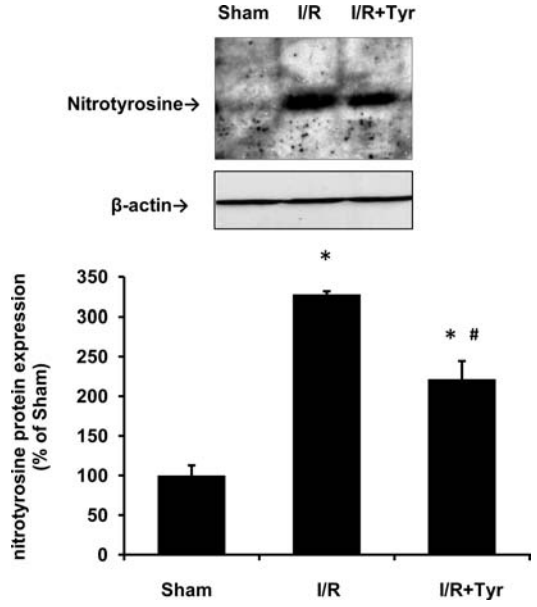

Figure 4. Effect of tyrosol treatment on nitrotyrosine-modified protein levels. The left kidney was subjected to sham operation (Sham), 45 min of ischemia followed by 6 h of reperfusion (I/R), or 45 min of ischemia followed by 6 h of reperfusion with tyrosol administration (100 mg/kg, intraperitoneal injection) 30 min prior to the induction of ischemia (I/R+Tyr). The histogram displays the relative density of nitrotyrosine-modified protein bands compared with β-actin. Results are expressed as the mean ± SEM (n = 5). ∗, $P < 0.05$ when compared with the value obtained from the sham-operated group; #, $P < 0.05$ when compared with the value obtained from the ischemia−reperfusion group.

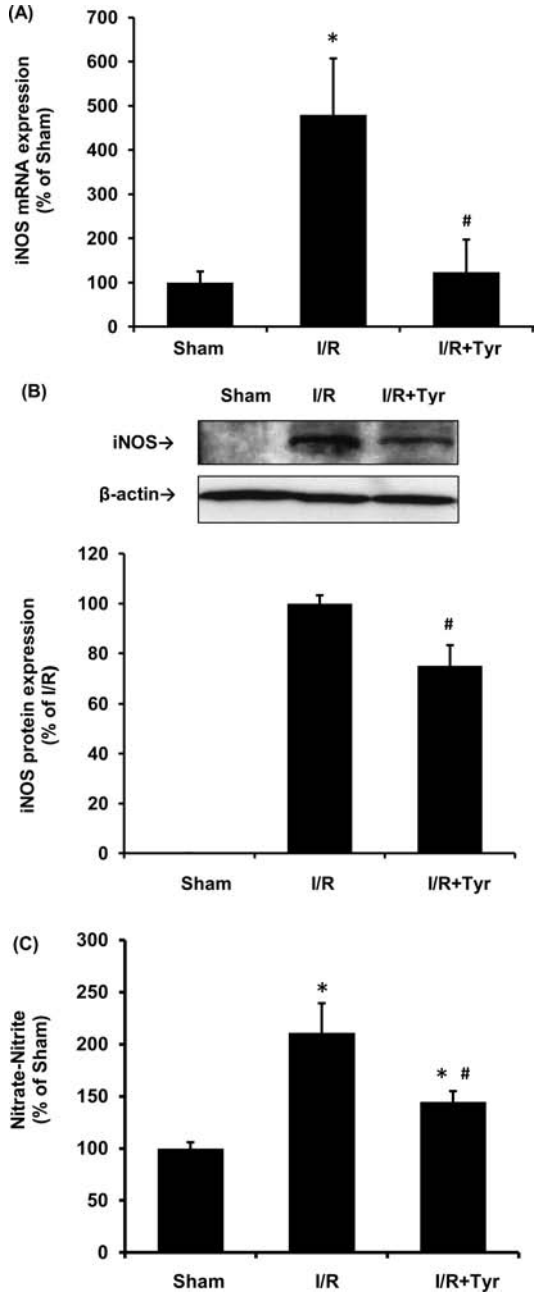

Figure 5. Effect of tyrosol treatment on iNOS expression and nitric oxide (NO) metabolites in the kidney tissue. The left kidney was subjected to sham operation (Sham), 45 min of ischemia followed by 6 h of reperfusion (I/R), or 45 min of ischemia followed by 6 h of reperfusion with tyrosol administration (100 mg/kg, intraperitoneal injection) 30 min prior to the induction of ischemia (I/R+Tyr). (A) iNOS mRNA in the kidney was determined by real-time PCR analysis. (B) iNOS protein was determined by Western immunoblotting analysis. (C) Kidney NO metabolites (nitrite and nitrate) were determined. Results are expressed as the mean ± SEM (n = 8). ∗, $P < 0.05$ when compared with the value obtained from the sham-operated group; #, $P < 0.05$ when compared with the value obtained from the ischemia−reperfusion group.

significant elevation of NO metabolite levels (nitrate and nitrite) in the kidney (Figure 5C). Administration of tyrosol effectively attenuated ischemia−reperfusion-induced elevation of iNOS mRNA and protein levels in the kidney tissue (Figure 5A,B). Such a treatment reduced the levels of NO metabolites (Figure 5C) in the kidneys. A similar inhibitory effect of tyrosol was also observed in the kidneys subjected to 45 min of ischemia followed by reperfusion for 1 h (data not shown). To investigate the mechanism by which tyrosol inhibited iNOS expression, nuclear proteins were prepared from the kidney tissue and EMSA was performed. Ischemia−reperfusion caused a significant increase in the NF-κB/DNA binding activity in the kidney tissue (Figure 6), which might lead to increased iNOS

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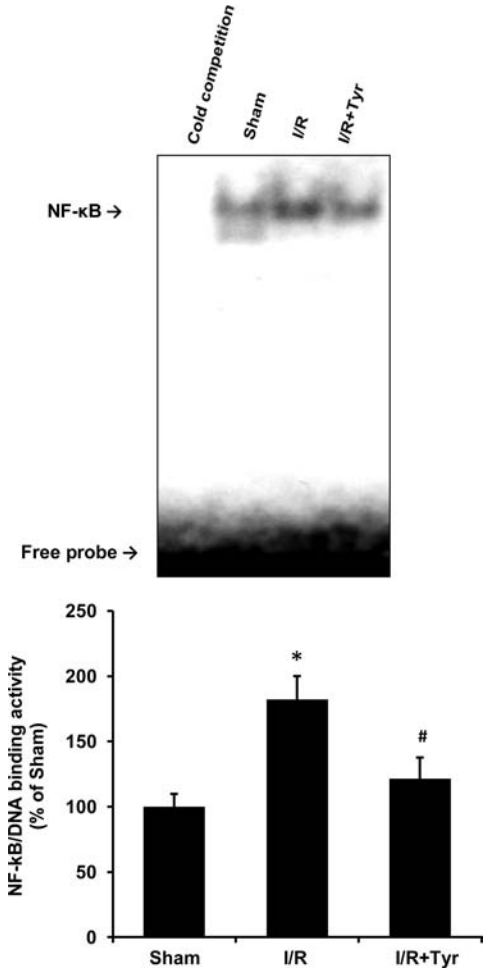

Figure 6. Effect of tyrosol treatment on the activation of NF-κB in kidney. The left kidney was subjected to sham operation (Sham), 45 min of ischemia followed by 1 h of reperfusion (I/R), or 45 min of ischemia followed by 1 h of reperfusion with tyrosol administration (100 mg/kg, intraperitoneal injection) 30 min prior to the induction of ischemia (I/R+Tyr). The DNA binding activity of NF-κB in the kidney was determined by EMSA. Results are expressed as the mean ± SEM (n = 5). *, $P < 0.05$ when compared with the value obtained from the sham-operated group; #, $P < 0.05$ when compared with the value obtained from the ischemia−reperfusion group.

expression. Administration of tyrosol effectively inhibited ischemia−reperfusion-induced NF-κB activation in the kidney tissue (Figure 6). These results suggested that tyrosol exerted an inhibitory effect on ischemia−reperfusion-induced iNOS expression through its inhibition of NF-κB activation.

DISCUSSION

The results obtained from the present study revealed that 45 min of ischemia followed by 6 h of reperfusion led to impaired kidney function in rats. Kidney dysfunction was accompanied by increased iNOS-mediated NO production and increased protein nitration and lipid peroxidation. The novel findings of the present study are that (1) administration of tyrosol prior to the induction of ischemia effectively reduced oxidative stress and improved the function of kidney subjected to ischemia−reperfusion and (2) such a beneficial effect of tyrosol was mediated, in part, through its inhibition of NF-κB activation and iNOS-mediated NO production.

NO plays an important role in physiological processes in the body. At physiological concentrations, NO regulates vasodilation and participates in cell signal transductions. However, activation of the iNOS isoform can lead to overproduction of NO, which is detrimental to tissues. The contribution of excessive NO to cell damage may be attributable to the effect mediated by the NO molecule itself or an indirect effect by peroxynitrite, the product of an interaction of NO with superoxide.[41] Studies in experimental animals have demonstrated that scavenging excessive NO or selective inhibition of iNOS can protect kidney from ischemia−reperfusion injury.[23,29,42] In the present study, ischemia−reperfusion caused a significant increase in iNOS expression in the kidney. This was accompanied by an elevation of NO metabolites in the kidney tissue. We previously reported that ischemia−reperfusion resulted in an activation of NF-κB.[43] This transcription factor regulates the expression of many inflammatory genes including iNOS.[43] Indeed, we observed a significant increase in NF-κB/DNA binding activity in the kidneys subjected to ischemia−reperfusion. Administration of tyrosol not only attenuated ischemia−reperfusion-induced NF-κB activation but also reduced iNOS expression in the kidney. In accordance, the levels of NO metabolites in the kidney were markedly reduced. This might account for a reduction of peroxynitrite formation in the kidney tissue.

Oxidative stress is characterized by an excessive accumulation of reactive radicals causing tissue damage. Peroxynitrite is a well-known reactive oxidant, and even a modest increase in peroxynitrite formation can result in extensive oxidation of macromolecules.[41] In the present study, up-regulation of iNOS expression led to excessive NO production in the kidney upon ischemia−reperfusion, which might contribute to an elevation of peroxynitrite formation and subsequently protein tyrosine nitration. This was accompanied by impaired kidney function. Histological examination revealed tubular necrosis and interstitial congestion in the kidney subjected to ischemia−reperfusion. Administration of tyrosol prior to the induction of ischemia significantly reduced nitrotyrosine levels and lipid peroxidation in the kidney. Such treatment also improved renal function and alleviated histological damage in the kidney subjected to ischemia−reperfusion. These results indicated that reduction of peroxynitrite formation by tyrosol treatment (100 mg/kg body weight, ip) might contribute to its beneficial effect against ischemia−reperfusion injury in the kidney. However, our results could not rule out the possibility that tyrosol at doses >100 kg/mg might exert further protective effects against ischemia−reperfusion injury. It should be noted that although olive oil phenolic compounds are well absorbed, it may not be feasible to achieve higher (pharmacological) concentrations of tyrosol (such as 100 mg/kg) in the circulation/organs through dietary intake of olive oil.

Oxidative stress has been identified as one of the important mechanisms that contribute to ischemia−reperfusion injury. Inhibition of oxidative stress by antioxidants has been shown to reduce ischemia−reperfusion-induced renal injury and improve renal function. Apart from attenuation of free radical overproduction, some antioxidants exert beneficial effects by direct scavenging of free radicals and/or restoring antioxidant enzyme activities. For example, ascorbic acid (vitamin C) exerts renal protective effects by direct radical scavenging.[44] Resveratrol, a phenolic compound, exerts its antioxidant effect via inhibition of tissue neutrophil infiltration as well as direct radical scavenging.[45] Another antioxidant, naringin, is an active

flavanone glycoside found in grapefruit. One of the mechanisms attributable to its antioxidant effect is through the restoration of antioxidant capacity by increasing superoxide dismutase and catalase activities.[46]

Although results from the present study demonstrated that tyrosol exerted a renoprotective effect against ischemia−reperfusion-induced oxidative stress via inhibition of iNOS-mediated NO production and peroxynitrite formation in the kidney, other phenolic compounds in olive oil such as hydroxytyrosol could also contribute to health benefits. Both tyrosol and hydroxytyrosol have been shown to have comparable bioavailabilities and exert antioxidant effects. It is plausible that hydroxytyrosol may have a similar or better renoprotective effect against ischemia−reperfusion injury via similar or different mechanisms. Investigation of renal effects of hydroxytyrosol and other dietary phenolic compounds is warranted in future studies.

Other phenolic compounds such as oleuropein, hydroxytyrosol, and caffeic acid are also present in olive pomace oil or olive oil in the same or even higher concentrations. The beneficial effects of tyrosol as a natural phenolic antioxidant along with other phenolic compounds have also been examined in other experimental models. For example, tyrosol, oleuropein, and polyphenol extract from olive pomace oil have been shown to reduce NO levels in human endothelial cells subjected to anoxia.[47] Another study has demonstrated that tyrosol and β-sitosterol (a minor compound in olive oil) are able to reduce PMA-induced NO releasefrom macrophages.[48] A recent study reports that tyrosol and salidroside (a bioactive compound found in *Rhodiola*) exert a protective effect against ischemia−reperfusion-induced apoptosis in cardiac cells.[49] Furthermore, studies also suggest that phenolic compounds such as caffeic acid, hydroxytyrosol, and oleuropein found in olive oil are able to attenuate inflammatory reactions and scavenge peroxynitrite.[11,50] Results from the present study provided novel evidence on the kidney-protective effect of tyrosol against ischemia−reperfusion injury. Our results suggested that regulation of NF-κB activation might be one of the mechanisms by which tyrosol attenuated ischemia−reperfusion-induced iNOS expression in the kidney.

In summary, the present study has demonstrated, for the first time, that tyrosol treatment attenuated iNOS-mediated NO production, which in turn reduced oxidative stress and minimized the extent of kidney injury induced by ischemia−reperfusion. Our results suggest that tyrosol may be considered as a potential natural health product for the prevention and treatment of ischemia−reperfusion-induced tissue injury such as acute kidney injury.

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Notes

The authors declare no competing financial interest.

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