The Protective Effect of Erdosteine on Radiocontrast Induced Nephrotoxicity in Rats

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ABSTRACT: Acute renal failure resulting from radiocontrast-induced nephrotoxicity (RIN) is suggested to occur via medullary ischemia coupled with the generation of free radicals and oxidative injury to tubular cells. The aim of the present study was to assess the effects of erdosteine on prevention of RIN. Thirty-three Wistar-albino rats were divided into five groups: control (group 1, $n = 6$), radiocontrast media (group 2, $n = 6$), erdosteine (group 3, $n = 7$), erdosteine four doses before radiocontrast application (group 4, $n = 7$) and erdosteine one dose at the same day with radiocontrast application (group 5, $n = 7$). RIN was induced by administration of intravenous high osmolar contrast media amidotrizoate (6 mL/kg). Total RNA was extracted from the kidney, and the expression levels of *Lipocalin 2 (Lcn2)* and *secreted phosphoprotein 1 (Spp1)* genes were evaluated by real time reverse transcription polymerase chain reaction (real-time RT-PCR). Total antioxidant status (TAS) and total oxidant status (TOS) were measured in kidney homogenates and serum samples. Serum creatinine, BUN (Blood Urea Nitrogen) and cystatin-C levels were measured from serum samples. The kidneys were evaluated histopathologically. The expression levels of *Spp1* and *Lcn2* genes in group 2 were significantly higher than groups 1, 3, 4, and 5. The expression levels of *Spp1* and *Lcn2* genes in group 4 were four and two times lower than group 5, respectively. Kidney TOS levels in group 2 were significantly higher than groups 1, 3, 4, and 5. Kidney TAS levels in group 3 were higher than group 2. Kidney oxidative stress index (OSI) levels in group 2 were significantly higher than groups 4 and 5. All rats in contrast media group developed tubular necrosis, proteinaceous casts, medullary congestion although these changes were significantly reduced in groups 4 and 5. This study demonstrated that multiple doses of erdosteine before application may have higher protective effects against RIN. Environ Toxicol 26: 395–402, 2011.

Keywords: erdosteine; renal toxicity; radiocontrast nephrotoxicity; *Lcn2*; *Spp1*; oxidative stress

INTRODUCTION

Radiocontrast nephrotoxicity (RIN) is the third common cause of acute renal failure (Waybill and Waybill, 2001; Agrawal and Stouffer, 2002). The RIN occurs in 1-6% of hospitalized patients and can reach up to 50% in patients with high risk such as renal insufficiency, diabetic nephropathy, volume depletion, dehydration, hypercholesterolemia, and old-age (Parfrey et al., 1989; Andrade et al., 1998; Waybill and Waybill, 2001). Although little is known about cellular mechanisms underlying radiocontrast nephropathy, two frequently proposed mechanisms were suggested to be

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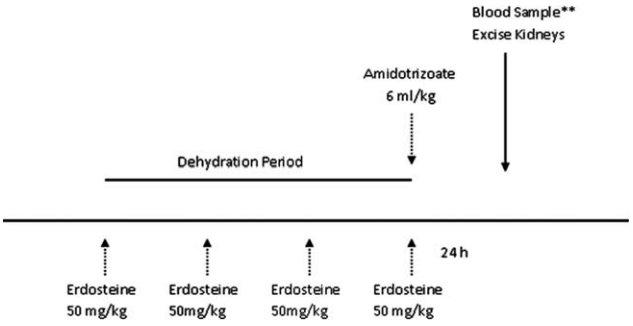

Fig. 1. The experimental procedure including drug application times.

involved in the pathogenesis of radiocontrast nephropathy; direct renal tubular epithelial cell toxicity mediated by oxygen free radicals and decrease in renal blood flow leading to renal medullary ischemia (Murphy et al., 2000; Curhan, 2003). Amidotrizoate sodium is an ionic, high osmolality radiocontrast substance. Although it is used in radiological applications, it has been associated with adverse side effects such as causing RIN, especially in high risk patients. It has been stated that the compounds with high-osmolality have more risk than low or iso-osmolar substance for nephrotoxicity (Rudnick et al., 1995).

In clinical examinations, some protective precautions and agents such as saline hydration, *N*-acetylcysteine (NAC), theophylline, calcium channel blockers, diuretics, dopamine, endothelin receptor antagonists, atrial natriuretic peptide, angiotensin-converting enzyme inhibitors, and prostaglandin E-1 have been used to prevent the RIN (Cox and Tsikouris, 2004). But, there is not any protective agent that has already been well-defined with widespread consensus (Arif et al., 2003). Erdosteine is a medical agent used to prevent the RIN (Ozyurt et al., 2004; Cabuk et al., 2008). However, studies on the protective effects of erdosteine from RIN are not sufficient (Moretti and Marchioni, 2007).

The aim of this study was to investigate the possible protective effects of erdosteine on RIN, and to compare benefits of erdosteine at different doses. The expression levels of *Spp1* and *Lcn2* genes, which are sensitive biomarkers of nephrotoxicity, were measured to show nephrotoxicity in early stages in conjunction with biochemical and histopathological evaluations (Wang et al., 2008).

Material Methods

The study protocol was approved by the Local Ethical Committee of Laboratory Animals at Ankara Numune Training and Research Hospital.

Animals and Treatment

Thirty-three female Wistar rats weighing 250–300 g were included in the study. The rats were randomly divided into five groups and maintained in a 12-h light-dark cycle. All rats were given unlimited access to standard rat chow and water excluding the 24-h period preceding the randomization procedure when all animals were deprived of water. The rats were randomized into five groups, as follows: group 1; controls ($n = 6$), group 2; radiocontrast media ($n = 6$), group 3; erdosteine ($n = 7$), group 4; erdosteine 4 doses + radiocontrast media ($n = 7$) and group 5; radiocontrast media + erdosteine 1 dose ($n = 7$). The rats tolerated and completed the treatment well except one from group 2/rat 2. Erdosteine and radiocontrast media were applied as shown by Figure 1.

At the end of the 4th day, the rats were anesthetized with an intramuscular injection of 50 mg /kg^{-1} ketamine hydrochloride and 8 mg/kg^{-1} xylazine, and then all rats were sacrificed. Synchronized anesthesia was administered to all five groups during administration of contrast medium. Blood sample was withdrawn from the abdominal aorta and serum samples for creatinine, BUN, and cystatin-C with serum total antioxidant status (TAS) and serum total oxidant status (TOS) measurements were kept frozen (-80°C) until the analysis. The kidneys were quickly removed, decapsulated, and divided equally into two longitudinal sections. One half was placed in formaldehyde solution for routine histopathologic examination. The other half of the kidney was washed with physiological saline and stored at -80°C for analysis of renal TAS and renal TOS. Another half of the kidney was divided into three to four pieces (20–30 mg) and reserved in RNAlater™ (Qiagen Inc., Valencia, CA), RNA stabilization reagent, at -20°C.

Molecular Genetics Evaluation

The damage of nephrotoxic agents in kidney routinely relies on the measurement of BUN and serum creatinine. However their relatively low sensitivity may hinder early detection of renal damage. Hence, the expression levels of *Spp1* and *Lcn2* genes were measured with real time RT-PCR method to show nephrotoxicity in early stages clearly.

One piece of the tissue in RNAlater™ were homogenized in a 2 mL-microcentrifuge tube by using disposable probes of TissueRuptor™ (Qiagen), and the total RNA from homogenized tissue was extracted with RNeasy Mini Kit™ (Qiagen) according to manufacturer's instructions in QiaCube™ (Qiagen) automatic DNA/RNA isolation robot. All RNA samples were measured with NanoDrop™ ND-1000 UV-Vis Spectrophotometer and kept frozen at -80°C after recording. Total RNA of 1 μg final concentration was converted to cDNA via the QuantiTect™ Reverse Transcription Kit (Qiagen) according to manufacturer's instructions in Corbett™ Palm-Cycler PCR (Qiagen). The cDNA were ran and visualized in 2% agarose gel.

The real time RT-PCR reaction mix was prepared with the QuantiTect™ SYBR Green PCR Kits according to manufacturer's instructions. The cDNA that were diluted at

concentrations of 1/100, 1/1000, 1/10,000, 1/100,000 was measured as duplicate in order to ensure reliability of the results. The PCR reactions were performed on the Rotor-Gene Q™ Real-Time PCR System (Qiagen) with the following conditions: 95°C for 15 s followed by 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 30 s. The PCR products were melted by increasing the temperature from 60 to 95°C, rising 0.5 or 1°C at each step, waiting 30 s on the first step then 5 s for each step thereafter The melting curve was performed between 70°C and 95°C rising 1°C, waiting 5 s for each step. The data of expression levels of *Spp1* and *Lcn2* genes were analyzed with the Rotor-Gene Q software version 1.7. Following primers were used to amplify the expression levels of *Lcn2* and *Spp1* genes at different doses of erdosteine, and *β-Actin* and *HPRT1* as housekeeping genes; *Lcn2*; forward: 5′-TCCGATGAACT GAAGGAGCG -3′, reverse: 5′-GAGGCCCAGAGAC TTGGCA -3′, *Spp1*; forward: 5′-GCACACAAGCA GACGTTTTGA -3′, reverse: 5′-CCGTCAGGGACATC GACTGT-3′, *β-Actin*; forward: 5′- CTATGAGGGTTAC GCGCTCC-3′, reverse: 5′- TAGTCTGTCAGGTCCCGG C-3′, *HPRT1*; forward: 5′- TTGCTCGAGATGTCATGA AGG-3′, reverse: 5′- CACACAGAGGGCCACAATG-3′.

Serum Creatinine, BUN, and Cystatin-C Measurements

Serum creatinine and BUN measurements were performed at once by the same technician with a Cobas Integra 400 plus (Roche Diagnostics) autoanalyser using original reagents and Creatinine Jaffe and Urease methods. Cystatin C levels were measured by a commercially available ELISA kit (Rat Cystatin C ELISA, BioVendor Research and Diagnostic Product, Czech Republic).

Renal and Serum TAS, TOS, and Oxidative Stress Index

After weighing, tissue samples and the kidneys were cut into small pieces. Renal tissues were homogenized in a 50 mM phosphate buffer solution (pH 7.4) using a homogenizer (Pro200 homogenizer) and then centrifuged for 10 min at 5.000 rpm.The upper clear part of the tissue homogenates was used for the measurements of TAS, TOS, and protein. All preparative procedures were performed at +4°C. Protein levels of the clear supernatants were determined by Lowry's method.

Total antioxidant status (Rel Assay Diagnostics, Turkey) and total oxidant status (Rel Assay Diagnostics, Turkey) were measured using supernatant fraction of homogenates and serum samples by using a commercially available Rel Assay Diagnostic kits. An autoanalyzer (Siemens Advia Chemistry System) was used.

Oxidative stress index (OSI) was calculated as TOS result divided by TAS.

Histopathological Evaluation

All kidney samples were sectioned and fixed on 10% formalin, dehydrated and embedded in paraffin. Tissues then were sectioned at 3μm and stained with hematoxylin and eosin (H&E), followed by semi quantitative analysis of the kidney sections by single pathologist using a blind protocol. Tubular necrosis and proteinaceous casts were graded as follows: no damage (− or 0), mild (± or 1, unicellular, patch isolated damage), moderated (+ or 2, damage less than 25%), severe (++ or 3, damage between 25 and 50%), and very severe (+++ or 4, more than 50% damage). The medullary congestion was defined as follows: no congestion (− or 0), mild (± or 1, vascular congestion with identification of erythrocytes by 400× magnification), moderate (+ or 2, vascular congestion with identification of erythrocytes by 200× magnification), severe (++ or 3, vascular congestion with identification of erythrocytes by 100× magnification) and very severe (+++ or 4, vascular congestion with identification of erythrocytes by 40× magnification).

Statistical Evaluation

Data were analyzed with SPPS 15.0 for windows program. The distributions of the each parameter within each group were evaluated by one sample Kolmogorov Smirnov test. The distributions were normal for each variable of all groups, so parametrical tests were used for analysis. One way ANOVA test was performed and post hoc multiple comparisons were done with LSD. P value less than 0.05 was the threshold for being statistically significant. The results were given as mean ± SEM.

RESULTS

The Evaluation of *Spp1* and *Lcn2* genes

The expression levels of *Lcn2* and *Spp1* genes in the groups were shown in Figure 2(a,b), respectively. The expression levels of *Lcn2* gene in group 3, 4, and 5 were similar to control group (group 1). However, *Lcn2* gene expression levels were significantly higher in group 2 than control group ($P < 0.028$), 3 ($P < 0.021$), 4 ($P < 0.030$), and 5($P < 0.045$) because the expression levels of *Lcn2* gene in group 2 was increased as ~22, 36, 7 and four-fold comparison to groups 1, 3, 4, and 5, respectively.

Although the expression levels of *Spp1* gene in groups 3 and 4 were similar to control group, its expression in group 2 were significantly higher than control group ($P < 0.006$), 3 ($P < 0.006$) and 4 ($P < 0.012$) because the expression levels of *Spp1* in group 2 was increased as ~43, 20, 7 and two-fold comparison to groups 1, 3, 4, and 5, respectively.

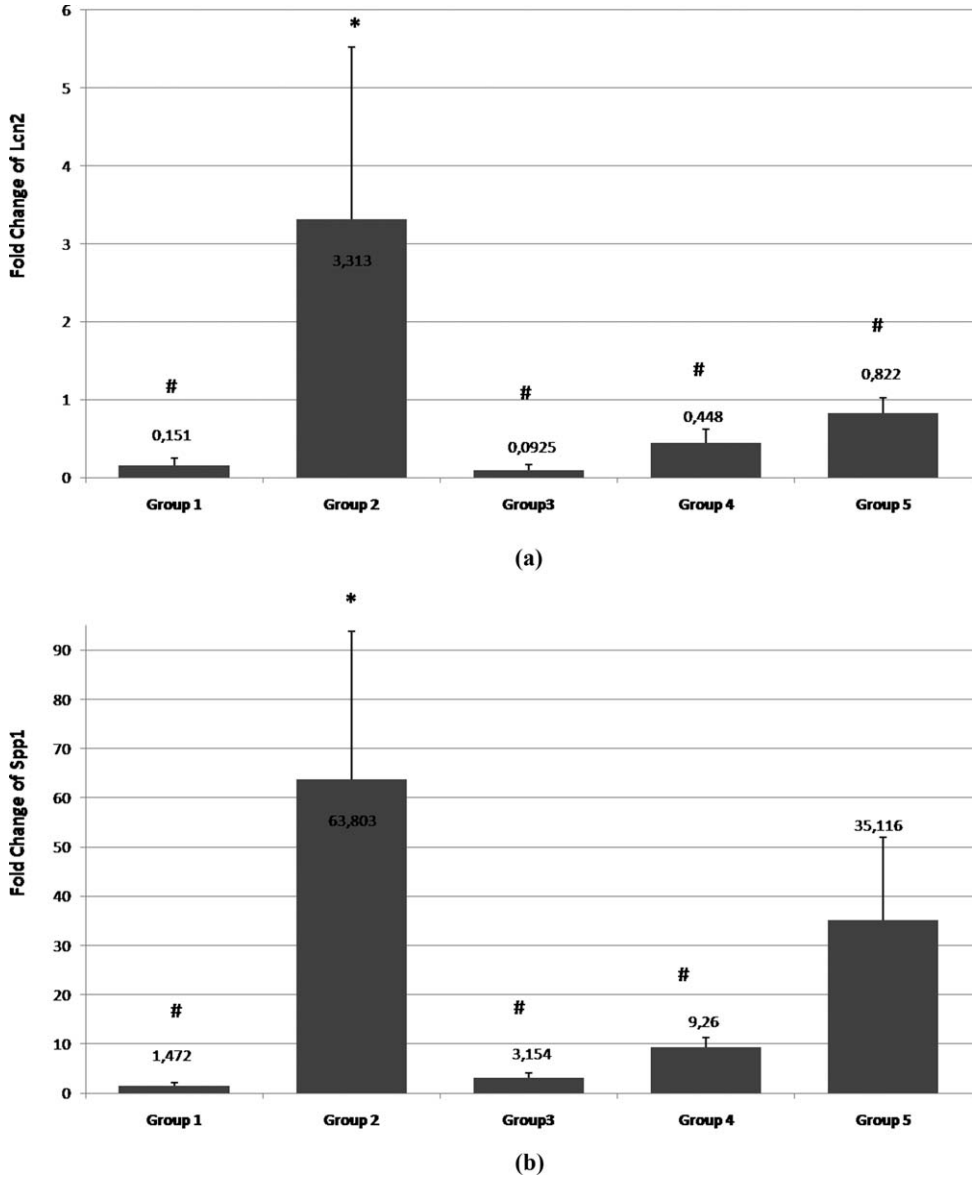

Fig. 2. The fold change of Lcn2 [Fig. 2(a)] and Spp1 [Fig. 2(b)] expressions levels were presented. Group 1; controls, group 2; radiocontrast media, group 3; Erdosteine for 4 doses, group 4; Erdosteine four doses before radiocontrast media application and group 5; Erdosteine one dose at the same day with radiocontrast media. $^{*}P < 0.05$ in comparison with group 1(control group). $^{\#}P < 0.05$ in comparison with group 2 (radiocontrast-treated group).

However, there was no significant difference the expression levels of *Spp1* genes between group 2 and 5, statistically.

Renal and Serum TAS, TOS, and OSI results

Kidney TOS levels in radiocontrast treated group (group 2) were significantly higher than those in groups 1, 3, 4, and 5 ($P = 0.002$, $P = 0.001$, $P = 0.039$, and $P = 0.026$, respectively) (Table I). Kidney TAS levels in control group were higher than those in all radiocontrast applied groups that were groups 2, 4, and 5 ($P = 0.003$, $P = 0.02$, and $P = 0.02$, respectively). Also, kidney TAS levels in group 3 were higher than in group 2 ($P = 0.025$). Kidney OSI levels in control group were significantly lower than those in groups 2, 4, and 5 ($P < 0.05$). Kidney OSI levels in group 2 were significantly higher than those in groups 4 and 5 ($P = 0.001$ and $P = 0.001$, respectively) (Table I).

Serum TOS levels in group 2 were significantly higher than those in groups 1, 3, 4, and 5 ($P < 0.001$, $P < 0.001$, $P = 0.001$, and $P = 0.026$, respectively). Similarly, serum TOS levels in groups 4 and 5 were significantly increased in comparison with in groups 1 and 3 ($P < 0.05$). Serum TAS levels in group 5 were significantly higher than in

TABLE I. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the kidney, serum samples as well as *P* comparison table

	Renal			Serum		
	TOS (μmol/mg prot)	TAS (μmol/mg prot)	OSI (%)	TOS (μmol H_2O_2 /L)	TAS (mmol Trolox /L)	OSI (%)
Group 1	1.33 ± 0.17	0.25 ± 0.03	5.37 ± 0.73	8.94 ± 0.94	0.60 ± 0.06	14.9 ± 2.37
Group 2	1.72 ± 0.15	0.18 ± 0.03	9.34 ± 1.39	21.18 ± 5.94	0.62 ± 0.14	34.11 ± 7.05
Group 3	1.30 ± 0.10	0.23 ± 0.02	5.66 ± 0.74	10.16 ± 1.30	0.66 ± 0.06	15.31 ± 1.74
Group 4	1.48 ± 0.25	0.20 ± 0.02	7.30 ± 1.25	14.16 ± 2.93	0.72 ± 0.09	19.52 ± 2.58
Group 5	1.46 ± 0.23	0.20 ± 0.04	7.26 ± 0.56	16.82 ± 3.34	0.74 ± 0.17	23.26 ± 4.80
P comparison table						
Group 1 vs. 2	0.002	0.003	0.000	0.000	NS	0.000
Group 1 vs. 3	NS	NS	NS	NS	NS	NS
Group 1 vs. 4	NS	0.02	0.001	0.009	NS	NS
Group 1 vs. 5	NS	0.02	0.002	0.000	0.049	0.001
Group 2 vs. 3	0.001	0.025	0.000	0.000	NS	0.000
Group 2 vs. 4	0.039	NS	0.001	0.001	NS	0.000
Group 2 vs. 5	0.026	NS	0.001	0.026	NS	0.000
Group 3 vs. 4	NS	NS	0.004	0.033	NS	NS
Group 3 vs. 5	NS	NS	0.005	0.001	NS	0.001
Group 4 vs. 5	NS	NS	NS	NS	NS	NS

Group 1; controls, Group 2; radiocontrast media, Group 3; erdosteine for 4 doses, Group 4; erdosteine for 4 doses plus radiocontrast media and Group 5, radiocontrast media-plus erdosteine for 1 dose.

group 1 ($P = 0.049$). Serum OSI levels in group 2 were significantly higher than those in groups 1, 3, 4, and 5 ($P < 0.001$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). Also, serum OSI levels in group 5 were higher than groups 2 and 3 ($P = 0.001$ and $P = 0.001$, respectively) (Table I).

Serum Creatinine, BUN, and Cystatin-C Results

Serum creatinine levels on day 4 were significantly higher in radiocontrast treated group than in controls and group 3 ($P = 0.02$ and $P = 0.01$, respectively). In addition to that, serum creatinine levels were significantly higher in group 4 than in 3 ($P = 0.04$) (Table II). Serum BUN levels on day 4 were significantly higher in group 5 than in 2 ($P = 0,.003$) (Table II).

Serum cystatin-C levels on day 4 were significantly higher in 2 than those in groups 1, 3, 4, and 5 ($P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.002$, respectively). Additionally, serum cystatin-C levels in group 4 were higher than 1 and 3 ($P = 0.001$ and $P = 0.001$, respectively). Serum cystatin-C levels were significantly higher in group 5 than those in groups 1, 3, and 4 ($P < 0.001$, $P < 0.001$, and $P = 0.03$, respectively) (Table II).

TABLE II. Serum creatinine, urea and cystatin-C levels of the study groups

	Serum Creatinine (mg/dL)	Serum Urea (mg/dL)	Serum Cystatin-C (mg/L)
Group 1 ($n = 6$)	0.29 ± 0.03$^{\#}$	46.1 ± 6.4	0.98 ± 0.02$^{\#}$
Group 2 ($n = 6$)	0.34 ± 0.02*	45.3 ± 7.4	1.99 ± 0.06*
Grouop 3 ($n = 7$)	0.28 ± 0.03$^{\#}$	49.4 ± 5.4	1.00 ± 0.05$^{\#}$
Group 4 ($n = 7$)	0.32 ± 0.04	53.1 ± 8.3	1.38 ± 0.07$^{\#}$
Group 5($n = 7$)	0.30 ± 0.03	54.2 ± 8.5$^{\#}$	1.62 ± 0.11$^{\#}$

$^{*}P < 0.05$ in comparison with Group 1(control group).

$^{\#}P < 0.05$ in comparison with Group 2 (radiocontrast treated group).

Group 1; controls, Group 2; radiocontrast media, Group 3; erdosteine for 4 doses, Group 4; erdosteine for 4 doses plus radiocontrast media and Group 5; radiocontrast media plus erdosteine for 1 dose.

Histopathological Results

The radiocontrast-treated rats had higher protein cast scores (1.17 ± 0.17) in comparison with control and erdosteine

TABLE III. The semiquantitative analysis of protein cast, medullary congestion and tubular necrosis in the groups

	Protein Cast	Medullary Congestion	Tubular Necrosis
Group 1	0.00 ± 0.00$^{\#}$	0.50 ± 0.22$^{\#}$	0.50 ± 0.22$^{\#}$
Group 2	1.17 ± 0.17*	2.33 ± 0.21*	1.17 ± 0.17*
Group 3	0.14 ± 0.14$^{\#}$	0.57 ± 0.20$^{\#}$	0.00 ± 0.00$^{\#}$
Group 4	0.29 ± 0.18$^{\#}$	1.29 ± 0.18$^{*\#}$	0.57 ± 0.20$^{\#}$
Group 5	0.57 ± 0.20$^{*\#}$	1.43 ± 0.20$^{*\#}$	0.29 ± 0.18$^{\#}$

$^{*}P < 0.05$ in comparison with group 1(control group).

$^{\#}P < 0.05$ in comparison with group 2 (radiocontrast treated group).

Group 1; controls, Group 2; radiocontrast media, Group 3; erdosteine for 4 doses, Group 4; erdosteine for 4 doses plus radiocontrast media and Group 5, radiocontrast media-plus erdosteine for 1 dose.

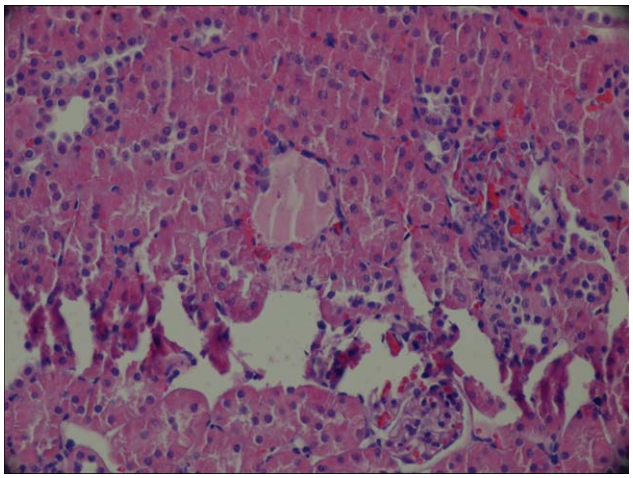

Fig. 3. Histopathological appearance indicating proteinaceous casts in the rats kidney from group 2, given just radiocontrast substance. (HE stain, ×200). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

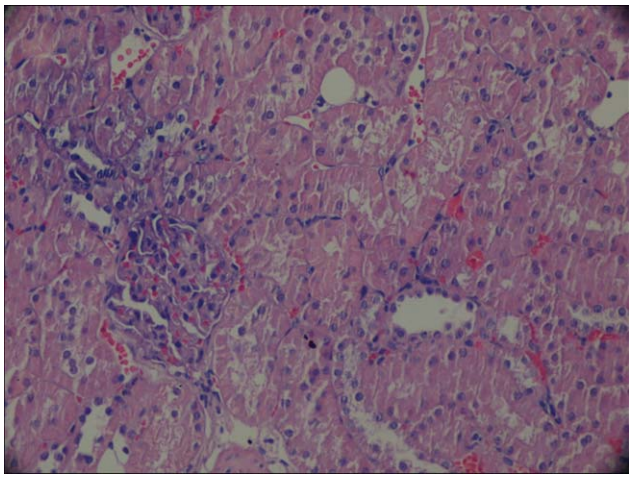

Fig. 5. Histopathological appearance indicating tubular necrosis in the rats kidney from group 2, given just radiocontrast substance. (HE stain, ×200). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

control groups ($P < 0.05$; Table III, Fig. 3). The erdosteine treatment before and after radiocontrast application (0.29 ± 0.18 and 0.57 ± 0.20, respectively) significantly reduced protein cast formation compared to group 2, which was a radiocontrast group ($P < 0.05$). However, the protein casts were significantly higher in group 5 than control group ($P < 0.05$).

The medullary congestions were significantly increased in group 2 (2.33 ± 0.21) in comparison with control (0.50 ± 0.22) and erdosteine control (0.57 ± 0.20) groups ($P < 0.05$; Table III, Fig. 4). The treatment with erdosteine before and after radiocontrast application decreased the medullary congestion significantly in comparison with group 2 (radiocontrast group) ($P < 0.05$; Table III, Fig. 4). However, the medullary congestion was not decrease as much as the control levels. The medullary congestion was higher in erdosteine treated before and after radiocontrast groups than control groups ($P < 0.05$; Table III, Fig. 4).

There were high score of tubular necrosis in radiocontrast group in comparison with control and erdosteine control groups ($P < 0.05$; Table III, Fig. 5). The groups 4 and 5 had lower tubular necrosis than group 2 (radiocontrast group) ($P < 0.05$; Table III, Fig. 5). There was no statistically significant difference between tubular necrosis scores of control and groups 4 or 5.

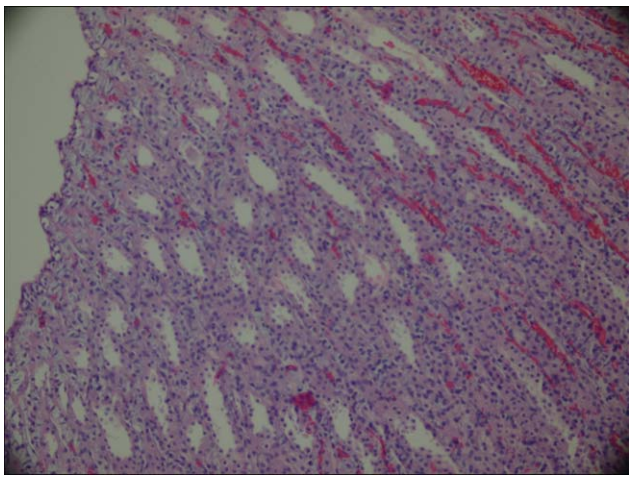

Fig. 4. Histopathological appearance indicating medullary congestion in the rats kidney from group 2, given just radiocontrast substance. (HE stain, ×100). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

In this study, we have evaluated whether erdosteine has protective effects on RIN. Erdosteine is a well-known mucolytic agent having antioxidant properties. We preferred erdosteine, because it is easily available, cheap and being used clinically. It plays also a role as a potential antioxidant and has mucolytic effects through its active metabolite as the reactive oxygen scavenging activity of erdosteine has been showed by many research groups (Braga et al., 2000; Fadillioglu and Erdogan, 2003). Moreover, clinical and experimental studies have demonstrated that erdosteine is a potent protective molecule against oxidative stress (Gurel et al., 2004). Hence, erdosteine seems to be a promising drug for the prevention of free radical-induced damage in many diseases.

Although decreased renal blood flow leads to renal medullar ischemia, the antioxidant features of erdosteine is more important to prevent nephrotoxicity since oxygen free radicals play an important role in etiology of RIN (Murphy et al., 2000; Curhan 2003).

In the study, oxidative stress was monitored by measuring of kidney and serum TAS and TOS levels. TOS concentration measurement of renal tissue and serum were revealed that radio-contrast media administration significantly increased both renal and serum oxidative stress as compatible with literature (Biagi et al., 1989; Vagliasindi and Fregman, 1989; Inglesi et al., 1994; Dechant and Noble, 1996; Braga et al., 2000). Erdosteine significantly attenuated the increase of TOS concentration in renal tissue and serum. Our study demonstrated that erdosteine such as *N*-acetylcysteine and ascorbic acid, produces antioxidant effects because erdosteine treatment against radiocontrast application in groups 4 and 5 had significantly reduced kidney OSI in comparison with radiocontrast group ($P < 0.05$). Furthermore, multiple erdosteine application seemed to be more effective according to OSI levels in the study.

Outer medullary congestion of the kidney is one of the vascular hallmarks of RIN because it is related to outer medullary hypoxia and hypoxic injury (Schrier et al., 2004). In our study, erdosteine treatment in group 4 and 5 could decrease the medullary congestion significantly in comparison with radiocontrast group ($P < 0.05$; Table III, Fig. 4) although the medullary congestion was not the same as control levels. Tubular obstruction resulted from protein cast is known as to be related with RIN. Although the protein casts was higher in group 5 than control group, erdosteine treatment with multiple doses may be attributed as having protective effect against RIN because protein casts in group 4 were significantly different from radiocontrast group as well as protein casts in group 5 was not higher than radiocontrast group (group 2). Furthermore, high score of tubular necrosis in radiocontrast group and low score in both of the erdosteine groups have also supported the preventive effect of erdosteine against RIN.

Spp1 gene is expressed in many tissues such as bone cells, inner ear, brain, kidney, placenta, and encodes a secreted glycoprotein called osteopontin. In fact, osteopontin is an important protein in many physiological and pathological processes including wound healing, bone turnover, tumorigenesis, inflammation, ischemia and immune responses since it interacts with multiple cell surface receptors (Wang and Denhardt, 2008). Osteopontin also plays an important role as antiapoptotic factor. It has been implicated in several renal pathological conditions such as those due to ureteral obstruction, ischemia, and toxic drugs (Wolak et al., 2009). It has been showed that the level of osteopontin may increase under nephrotoxic conditions, and *Spp1* gene expression may become a reliable biomarker for nephrotoxicity (Khan et al., 2002; Amin et al., 2004; Davis et al., 2004; Wang et al., 2008). In our study, the significant difference between radiocontrast media group (group 2) and groups 4 and 5 suggested the protective effect of erdosteine. Additionally, the results of this study also indicated that multiple doses of erdosteine before radiocontrast media have more benefits than single dose because the expression levels of *Spp1* and *Lcn2* genes in group 4 approximately six- and twofold lower than group 5, respectively. The higher protective effect of erdosteine in group 4 may depend on its cumulative antioxidant capacity. The histopathological evaluation with regard to tubular necrosis also supported this result (Fig. 3). Furthermore, increased expression levels of *Spp1* may depend on antiapoptotic features since oxygen-free radicals can induce the apoptosis (Jii et al., 2004).

Lcn2 is a member of the lipocalin superfamily. The product of *Lcn2* plays a role as a cellular iron carrier protein, which can induce the conversion of rat renal progenitor cells into epithelia, tubules, and complete nephrons (Yang et al., 2002). It has been showed that the expression levels and accumulation of *Lcn2* was increased in mouse and human kidney cortical tubules, as well as in blood and urine after ischemic and drug induced kidney injury (Mishra et al., 2005). In our study, the expression level of *Lcn2* was highly increased in radiocontrast group as 22-fold in comparison to control group. The expression level of *Lcn2* gene in group 4 was twofold lower than group 5 which was suggested that multiple doses of erdosteine application has more benefits than single dose.

In conclusions, the radiological tests are frequently used with a radiocontrast analysis. Thus, prevention of the radiocontrast toxicity is an important problem for clinicians since it is related to prolonged hospitalization, greater morbidity, health care costs and mortality. To decrease the risk of RIN one should especially avoid high osmolar and high volume of contrast media and nephrotoxic drugs and perform hydration with erdosteine, *N*-acetylcysteine, saline, ascorbic acid, and sodium bicarbonate treatments. Although there is a requirement for further studies to show exact mechanism of preventive effects of erdosteine treatment, we may suggest multiple doses of erdosteine could prevent radiocontrast nephrotoxicity according to our molecular, biochemical, and histopathological evaluations in rats.

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