Original Articles

Renal Function and Oxidative Stress following Gentamicin induced Renal Injury in Rats Treated with Erythropoietin, Iron and Vitamin E

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Abstract

The effects of erythropoietin(Epo), iron, and vitamin E on renal function and oxidative stress in rats with renal injury induced by gentamicin were investigated. Rats were divided into 5 groups; group 1, control; group 2, gentamicin group(100 mg/kg gentamicin sc. on days 5-12); group 3, gentamicin plus Epo (in addition to gentamicin, 100 i.u./kg Epo was administered s.c. on days 5-12); group 4, gentamicin plus Epo and iron (in addition to gentamicin and Epo, 500 mg/kg iron was administered i.p. on days 4) and group 5, gentamicin plus Epo, iron, and vitamin E (in addition to genta, Epo and iron, 250 i.u./kg, vitamin E was added orally on days 1-3) Renal functions and oxidative stress were investigated on days 12. The results show that glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) decreased in group 2. The urinary excretion of Na^+, K^+, protein and NAG increased in groups 2 to 5. GFR and ERPF were aggravated in group 3 with increased catalase (CAT) and superoxide dismutase (SOD) activities. Renal function and oxidative stress were unchanged in group 4 but higher ERPF with decreased BUN were found in group 5. It is concluded that gentamicin caused severe damage of both glomerular and tubular parts with alteration of oxidative stress. Epo and iron did not alter renal function but vitamin E supplementation could improve blood flow to the kidney.

Keywords : erythropoietin, gentamicin, iron, oxidative stress, renal function, vitamin E

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บทคัดย่อ

ผลของสารอิริทรอพอยอิทิน เหล็ก และวิตามินอี ต่อการทำหน้าที่ของไตและความเครียดออกซิเดชั่น ในหนูที่มีความเสียหายของไตจากการได้รับเยนตามัยซิน

พัชรินทร์ ธงไชย ณรงค์ศักดิ์ ชัยบุตร ชลลดา บูรณกาล*

ทำการศึกษาผลของสารอิริทรอพอยอิทิน (Erythropoietin; EPO) เหล็ก (iron) และวิตามินอี (vitamin E) ต่อการทำหน้าที่ของไตและความเครียดออกซิเดชั่น (oxidative stress) ในหนูที่มีความเสียหายของไตจากการได้รับเยนตามัยซิน (gentamicin) แบ่งหนูออกเป็น 5 กลุ่ม กลุ่มที่ 1 กลุ่มควบคุม กลุ่มที่ 2 ได้รับ gentamicin ขนาด 100 มิลลิกรัม/กิโลกรัม ฉีดเข้าใต้หนังในวันที่ 5-12 กลุ่มที่ 3 ได้ gentamicin ร่วมกับการฉีด EPO ขนาด 100 ยูนิต/กิโลกรัม เข้าใต้หนังในวันที่ 5-12 กลุ่มที่ 4 ได้ gentamicin ร่วมกับ EPO และ iron โดยให้ในขนาด 500 มิลลิกรัม/กิโลกรัมเข้าช่องท้องครั้งเดียวในวันที่ 4 กลุ่ม 5 ได้รับ gentamicin, EPO, iron และ vitamin E โดยให้กินในขนาด 250 ยูนิต/กิโลกรัม ในวันที่ 1-3 ผลการทดลองพบว่า อัตราการกรองผ่านกลอเมอรูลัส (glomerular filtration rate; GFR) และ อัตราการไหลของพลาสมาผ่านไต (effective renal plasma flow; ERPF) ลดลงในกลุ่มที่ 2 การขับทิ้งโซเดียม โพแทสเซียม โปรตีน และเอนไซม์ NAG เพิ่มขึ้นในกลุ่มที่ 2-5 พบว่า GFR และ ERPF ลดลงในกลุ่มที่ 3 โดยมีการเพิ่มขึ้นของการทำงานของเอนไซม์คาทาเลส (catalase) ซูเพอร์ออกไซด์ดิสมิวเทส (superoxide dismutase) ในกลุ่ม 4 การทำงานของไตและความเครียดออกซิเดชั่นไม่เปลี่ยนแปลงในขณะที่กลุ่ม 5 มี ERPF เพิ่มขึ้นและ ยูเรียไนโตรเจนในเลือดลดลง การทดลองนี้สรุปได้ว่า gentamicin ทำให้ไตเสียหายทั้งในส่วนกลอเมอลูลัส และหลอดไตฝอย และมีการเปลี่ยนแปลงความเครียดออกซิเดชั่น ทั้ง EPO และ iron ไม่มีผลต่อการทำหน้าที่ของไตแต่ vitamin E เพิ่มเลือดไปเลี้ยงไต

คำสำคัญ : อิริทรอพอยอิทิน เยนตามัยซิน เหล็ก ความเครียดออกซิเดชั่น การทำหน้าที่ของไต วิตามินอี

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Introduction

Gentamicin, aminoglycoside antibiotic, although has a good antimicrobial activity, has seriously adverse effects notably acute tubular necrosis (Mazzon et al., 2001). Nephrotoxicity is manifested by increased concentrations of plasma urea nitrogen (PUN), serum creatinine and urinary brush border enzymes(N-Acetyl-β-D-glucosaminidase; NAG and gamma glutamyl transferase; γ-GT). Previous study showed that recombinant human Erythropoietin (Epo) improved both anemia and renal function in gentamicin-treated rats (Nagano et al., 1990). Epo is considered as a growth factor by accelerating tubular cell regeneration (Bagnis et al., 2001). Epo decreased plasma levels of lipid peroxidation and malondialdehyde (MDA) in CRF patients (Sommerburg et al., 2000). Accelerated red blood cell production using Epo requires iron supplementation. However, iron promotes oxidative stress by catalyzing the Fenton reaction (Lim and Vaziri, 2004) leading to glomerulosclerosis, tubular atrophy, interstitial fibrosis and renal failure (Zhou et al., 2000). Thus, giving iron may aggravate renal function and vitamin E should be supplemented. The combined effects of Epo, iron and vitamin E on renal function and oxidative stress in gentamicin induced nephrotoxicity has not yet been demonstrated. The aims of this study were to investigate; first, to study effects of Epo administration on renal function and oxidative stress in rat with gentamicin-induced renal injury, second, to study effects of Epo, iron, vitamin E and their combinations on renal functions and oxidative stress.

Materials and Method

Experimental Animals: The experiment was performed in accordance with the institutional guidelines and conformed to the Faculty of Veterinary Science, Chulalongkorn University. Male Spraque-Dawley rats, weighting between 250-300 g obtained from National Laboratory Animal Center (NLAC), Thailand, were used. The animals were housed under standard conditions of light and dark cycle (L:D=12:12) with free access to rat chow and water. The animals were randomly assigned into 5 groups. Group 1 (control group, n=14) rats were injected with normal saline solution (NSS) subcutaneously to replace gentamicin and Epo on days 5 to 12 and intraperitoneal to replace iron dextran on days 5. Propylene glycol was fed once daily on days 2 to 4 to replace vitamin E. Group 2 to 5 were receiving gentamicin sulfate subcutaneously at a dose of 100 mg/kg for 8 days(day 5-12). Group 2(gentamicin groups, n=16) rats were injected with gentamicin sulfate alone. Group 3(gentamicin+Epo, n=16) rats were daily injected with Epo100 IU/kg subcutaneously in addition to gentamicin starting from day 5 until day 12. Group 4 (gentamicin+ Epo+iron, n=13) rats were injected with a single dose of 500 mg/kg iron dextran intraperitoneally on day 5 in addition to gentamicin and Epo. Group 5 (gentamicin+ Epo+iron+vitamin E, n=14), rats were fed once daily with 250 mg/kg vitamin E for 3 consecutive days prior to gentamicin administration (day 2-4) in addition to gentamicin, Epo and iron.

Body weight and food intake were recorded daily throughout the study. On the first day of experiment, each animal was kept in metabolic cage. Urine was collected for measurement of urine volume and concentrations of protein, electrolytes, malondialdehyde (MDA) and osmolarity and while plasma was collected by cutting tip of tail vein for measurement of plasma urea nitrogen (PUN) and creatinine concentrations. Repeated measurements were performed on days 11 with additional measurements of electrolyte (Na^+, K^+, Cl^-) concentrations. Renal clearance study was performed in 7 to 9 rats in each group on days 12. Urine was collected directly from urinary bladder before clearance procedure for measurement of urinary NAG activity. Blood was collected by cardiac puncture for measurement of concentrations of creatinine, PUN, electrolytes (Na^+, K^+, Cl^-) and osmolality. Six to eight separated rats from each group were used to study oxidative stress without renal function study. Rats were anesthetized and left kidneys were removed immediately for measurement of lipid peroxidation. The cortex of kidney was homogenated and stored at -70°C to determine concentration of MDA and activities of Superoxide Dismutase (SOD) and Catalase (CAT).

For renal clearance study, each rat was anesthetized by intraperitoneal injection with 50 mg/kg body weight of Tiletamine-Zolazepam (Zoletil®, Carros, France). The GFR and ERPF were determined using inulin and para-aminohippurate (PAH), respectively. The procedures and calculation for renal clearance and blood pressure were performed as previously described (Buranakarl et al., 2003) except the urine was collected from catheter passing into the urinary bladder.

Determinations of blood, urine and tissue samples

Inulin and PAH concentrations were determined by the antrone method (Young and Raisz, 1952) and method of Brun (1951), respectively. Sodium (Na^+) and potassium (K^+) were measured by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA). Chloride (Cl^-) was measured by chloridometer (Chloride analyzer 925, ciba Corning Inc., USA). Osmolarity was measured by osmometer (Osmometer 3D3, Advance Instruments Inc., USA). Urine protein concentration was measured by precipitating with sulfosalicylic while kidney protein concentration was measured according to Lowry et al. (1951). The creatinine concentration was analyzed by Jaffe reaction. The PUN concentration was analyzed by the colorimetric method. Urine and kidney MDA was assayed in the form of thiobarbituric acid reacting substances (TBARS) as described by Ohkawa

et al.(1979). Kidney CAT was determined following the method of Aebi(1983). Kidney SOD activity was measured following the method of McCord and Fridovich (1969). Urinary NAG was measured by enzymatic method.

Statistical analysis: Data were expressed as mean ± S.E. Paired t-test was used to compare data in the same group before and after treatment. The data among groups were compared with one-way ANOVA or one-way ANOVA on rank and post-hoc analysis with Student Newman-Keuls or Dunn methods to compare the data in pairwises. Differences between mean were considered significant at p less than 0.05. The Sigma-Stat program was used for statistical analysis.

Results

Food intake and body weight:

Food intakes in all groups receiving gentamicin (2 to 5) were declined starting on days 8. On days 10, food intake in all treatment groups were decreased significantly compared with group 1 which was correspond a significant reduction in body weight found in group 4 and 5.

Plasma creatinine and urea nitrogen (PUN) concentrations:

Group 3, 4, and 5 had significantly higher plasma creatinine concentrations on days 11 ($p< 0.01$) compared with day 1. The PUN concentrations on day 11 showed a similar results which were significantly higher in all groups that received gentamicin (Fig. 1). By comparing among groups, group 3 and 4 had the highest concentrations of both creatinine and PUN and significantly differences than control group 1 ($p<0.05$). In group 5, both creatinine and PUN concentrations tended to be lower than group 3 and 4.

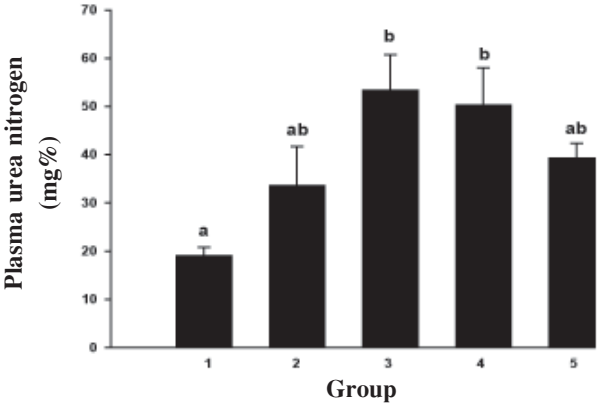

Figure 1 The plasma urea nitrogen concentrations in 5 groups of rats. The data are shown as mean ± S.E.[a,b] Means with different superscripts differ significantly between groups ($p<0.05$).

Renal hemodynamics

The GFR was significantly lower in group 2, 3, 4 and 5 as compared with group 1 (Fig. 2). However, only group 3 and 4 had significantly decrease in ERPF. Group 5 tended to increased ERPF compared with group 2, 3 and 4. High FF and RVR were found in group 2, 3 and 4 but the significant of RVR was found only in group 3 compared with group 1. The FF was decreased in group 5. Mean arterial blood pressures(MABP) were not different among groups (87±5, 86±3, 93±2, 95±7 and 92±4 mmHg in group 1, 2, 3, 4 and 5, respectively).

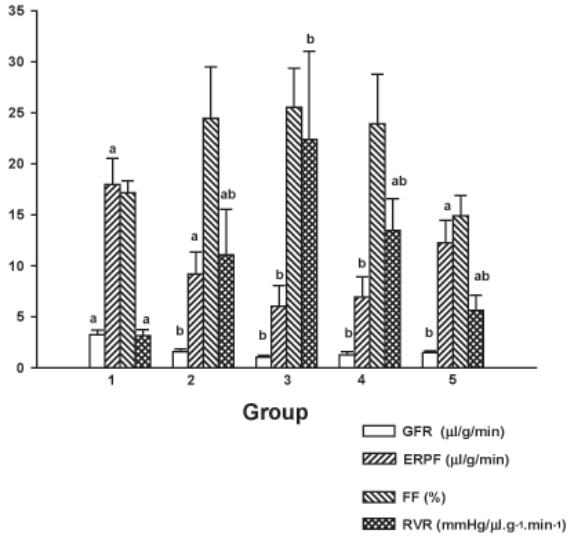

Figure 2 Renal hemodynamics in 5 groups of rats. The data are shown as mean±S.E.[a,b] Means with different superscripts differ significantly between groups ($p<0.05$).

The packed cell volume (PCV) only in group 2 had a significantly lower PCV (39.1±0.7%) compared with day 1 (49.2±2.3%) (p<0.05). No significant differences were found in plasma concentrations of Na^+, K^+, Cl^- and osmolarity in all groups at days 12.

Tubular function

Rats in groups 2, 3, 4 and 5 had significantly higher of the urine flow rate as compared with group 1 (Table 1). At day 12, increase in both fractional and urinary excretion of Na and K were found compared to group 1. There were no significant differences of fractional excretion of Cl and C_{osm} among groups. Free water clearance was lower in groups 2, 3, 4 and 5 (p<0.05) compared with group 1. The urinary protein excretion and UPC ratio were significantly higher in group 2, 3, 4 and 5 compared with before treatment (p<0.001). When comparing among groups, group 2, 3, 4 and group 5 had higher protein excretion and UPC ratio as compared with group 1. The urinary NAG/creatinine ratio was significantly increased in groups 2, 3, 4 and 5 as compared with group 1.

Oxidative stress

No significant differences of urinary MDA excretions were found among groups both on day 1 and days 11. Also, no significant change of kidney MDA was found in all groups (Fig. 3). The CAT activity was significantly higher in group 2, 3, 4 and 5 while SOD activity was higher in all 4 groups but the significance was found only in groups 3, 4 as compared with group 1.

Discussion

Gentamicin induced nephrotoxicity is characterized by direct acute tubular necrosis, primarily localized to the proximal tubule(Cuzzocrea et al., 2002). The tubular damage was demonstrated by decreased urine concentration capacity, proteinuria, enzymuria, and ultrastructural alteration of glomerular and tubular cell. The alteration of hemodynamics associated with

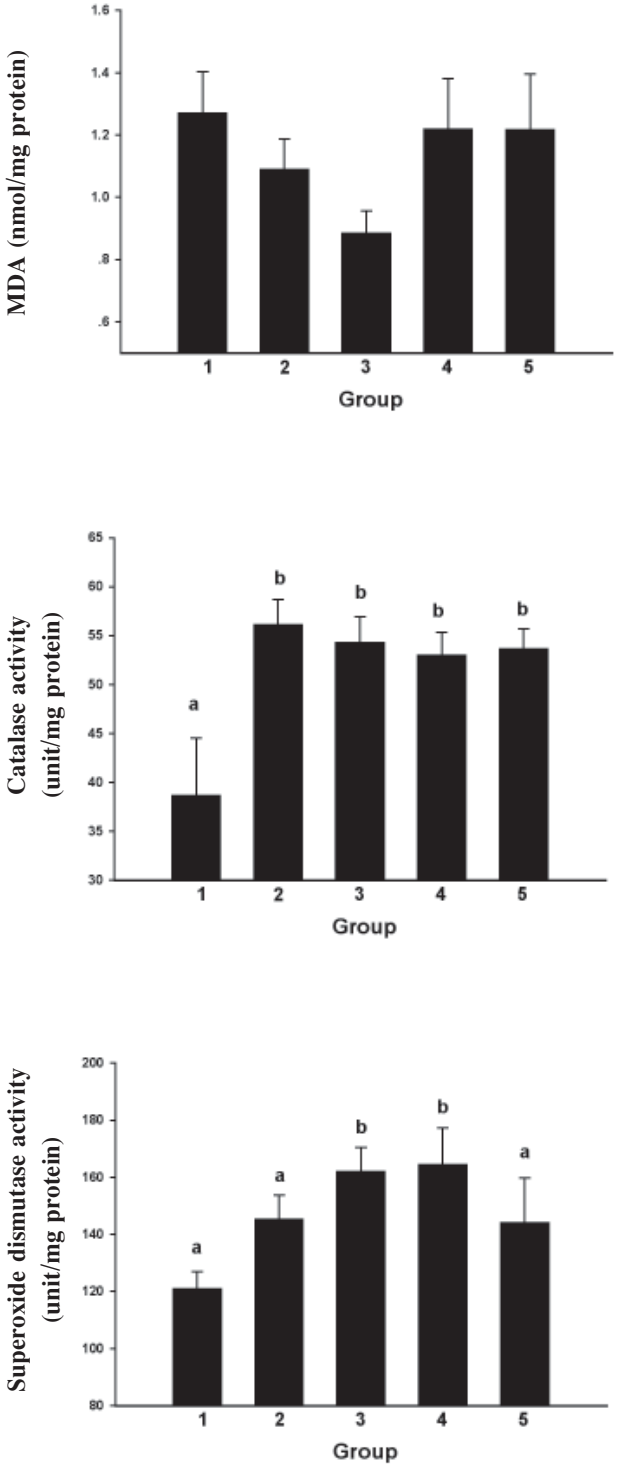

Figure 3 Oxidative stress in 5 groups of rats. The data are shown as mean ± S.E.[a,b] Means with different superscripts differ significantly between groups (p<0.05).

Table 1. Tubular function in all group

Parameter	group 1 (n=14)	group 2 (n=14)	group 3 (n=8)	group 4 (n=13)	group 5 (n=14)
Urine flow rate (ml/day)	14.91 ± 2.19[a]	26.42 ± 2.65[b]	29.86 ± 3.06[b]	22.11 ± 2.64[b]	27.07 ± 2.30[b]
FE_{Na+} (%)	3.37 ± 0.49[a]	6.56 ± 1.20[ab]	12.18 ± 1.53[b]	9.46 ± 1.38[b]	11.07 ± 1.46[b]
FE_{K+} (%)	2.64 ± 0.31[a]	6.48 ± 1.12[ab]	13.14 ± 2.19[b]	9.69 ± 1.30[b]	10.61 ± 1.29[b]
$U_{Na}V$ (mEq/day)	6.511 ± 0.920[a]	9.351 ± 0.852[b]	9.858 ± 0.551[b]	8.218 ± 0.535[ab]	8.815 ± 0.746[ab]
$U_{K}V$ (mEq/day)	0.213 ± 0.026[a]	0.355 ± 0.032[b]	0.379 ± 0.020[b]	0.315 ± 0.019[b]	0.325 ± 0.027[b]
$U_{Cl}V$ (mEq/day)	0.563 ± 0.093[a]	0.418 ± 0.046[a]	0.345 ± 0.060[ab]	0.212 ± 0.023[bc]	0.289 ± 0.037[ac]
C_{H2O} (ml/day)	-26.04 ± 3.74[a]	-16.13 ± 3.59[b]	-10.76 ± 2.84[b]	-11.99 ± 3.24[b]	-10.14 ± 3.25[b]
$U_{prot}V$ (mg/day)	1.145 ± 0.159[a]	6.488 ± 0.499[b]	7.977 ± 0.137[b]	7.176 ± 0.773[b]	8.067 ± 0.898[b]
UPC ratio	0.138 ± 0.016[a]	0.568 ± 0.04[b]	0.766 ± 0.037[b]	0.721 ± 0.078[b]	0.805 ± 0.04[b]
U_{NAG}/creatinine (milliunit/mg creatinine)	1.08 ± 2.20[a]	25.87 ± 4.58[b]	29.90 ± 2.34[b]	21.73 ± 2.50[b]	29.89 ± 3.52[b]

The data are shown as mean ± S.E. [a,b]Means with different superscripts differ significantly between groups, (p<0.05) by using one way ANOVA Number in parenthesis indicated number of animals.

disproportional less reduction in GFR than in renal plasma flow which was found in group 2, 3, and 4 indicating glomerular hyperfiltration phenomenon. This is due to preferential renal efferent arteriole vasoconstriction. The possible mechanisms of vasoconstriction by gentamicin is due to the releasing of vasoconstrictors from activated renin angiotensin system, the increasing renal endothelin content or inhibiting the production of vasodilatory prostaglandin PGE_2 (Assael et al., 1985; Hishida et al., 1994). In addition, Baylis and co-workers (1977) found many abnormalities of renal circulation such as renal vasoconstriction and reduction of glomerular capillary ultrafiltration coefficient(Kf) resulting in reduced GFR.

Gentamicin increased urinary excretions of Na, K, protein and NAG. The polycationic aminoglycosides may affect several processes involved in renal handling of lysozyme including glomerular permeability, tubular reabsorption and intracellular proteolytic degradation resulting in increased NAG (Cojocel et al., 1983). Proteinuria of glomerular and/or tubular origin is an index of gentamicin nephrotoxicity (Cojocel et al., 1984).

Gentamicin enhanced generation of superoxide anion and hydrogen peroxide and causing renal vasoconstriction (Nagajima et al., 1994). Administration of M40403, a superoxide dismutase mimetic, attenuated the effect induced by gentamicin (Cuzzocrea et al., 2002). In the present study, although the activities of antioxidant enzymes were increased, oxidative damage may not occur since no changes of both kidney and urinary MDA excretion were found. Similary, Fauconneau and coworkers (1995) showed no modification of TBARS in rats received gentamicin. The results were supported by previous study which showed that gentamicin induced increase in superoxide production and SOD activity but did not induce detectable changes in membrane fluidity and lipid peroxidation (Soldago et al., 2002). In contrast, Ramsammy and coworkers (1985) reported that gentamicin injection at the dose of 100 mg/kg/day for 1 to 4 days induced lipid peroxidation in rat renal cortex. Yamada (1995) was also found increasing of renal MDA after administration of gentamicin at a dose of 120 mg/kg subcutaneously for 7 consecutive days. The differences in the result of many investigators may involve the differences in experimental designs regarding dose and duration of gentamicin administration.

When rats received Epo, the PCV was increased. However, Epo did not improve but further reduce ERPF and GFR. Hyperfiltration was still maintained in this group. The mechanism of increasing in RVR may be a result of endothelin-mediated vasoconstriction by Epo receptor on the surface of vascular endothelial cell of the renal circulation (Slowinski et al., 2002). Moreover, RVR may be high due to blood viscosity after PCV was elevated. Epo can also aggravate glomerular injury and promoted hypertension in rats (Garcia et al., 1988). However, no hypertension was found in the present study which may be due to natriuresis and diuresis activated by tubular endothelial system (Marex et al., 1999). Endothelin-1 (ET-1) inhibit the activity of the epithelial sodium channel via ET_β receptor, leading to a decrease in Na and water reabsorption in the renal tubule (Ohuchi et al., 2000; Hocher et al., 2001). Thus, enhanced Na^+ and K^+ excretions were found in group 3, 4 and 5 which received Epo. From the results, Epo did not have cytoprotective effect but rather worsen renal functions.

In group 4, renal hemodynamics and tubular functions and antioxidant enzymes activities were similar to group 3. Thus, iron did not aggravate renal dysfunction. Zager and coworkers (2004) demonstrated that iron sucrose had the most renal tubular toxicity, while iron dextran caused the highest oxidative stress. Agarwal et al. (2004) suggested that intravenous iron produced oxidative stress and increasing urinary excretion of protein and MDA. However, no alterations of both protein and MDA excretions compared with group 3 were found in the present study.

Vitamin E increased ERPF by vasodilation resulting in the decreased in FF. The PUN and creatinine concentrations were declined compared with group 2, 3

and 4. Previous study showed that vitamin E pretreatment before gentamicin could lower BUN and creatinine (Abdel-Naim et al., 1999). It was suggested that vitamin E induced increase in PGI_2 and PGE_2 production (Wu et al., 2005).

Vitamin E did not improve tubular function. The urinary electrolyte, protein and NAG excretions were still high. Kidney CAT was high although SOD seems to be declined similar to the previous study (Ibrahim and Chow, 2005). Pretreatment with vitamin E for six days prior gentamicin treatment caused decline in renal cortical MDA, GSH, SOD and CAT in rats receiving gentamicin (Ramsammy et al., 1987). Moreover, vitamin E was effective in controlling iron dextran induced radical generation in the kidney (Galleano et al., 1994). Thus, vitamin E can improve blood flow and reduced oxidative damage.

In conclusion, the present study demonstrates that gentamicin had direct effect on hemodynamic, tubular function and oxidative stress by ameliorated GFR and ERPF, increased urine flow rate, urinary excretion of Na^+, K^+, water, protein and lysosomal enzyme (NAG). Epo administration did not improve renal function while supplement with iron together with Epo caused no further damage. Supplementation of vitamin E did not correct tubular damage but increased renal plasma flow and reduced oxidative damage.

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