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ORIGINAL PAPER

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Protective effect of aminoguanidine against nephrotoxicity induced by amikacin in rats

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Abstract Aminoglycoside antibiotics have long been used in antibacterial therapy. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic and ototoxic side effects. It has been reported that reactive oxygen radical species (ROS) play role in the pathophysiology of aminoglycosides-induced nephrotoxicity. Aminoguanidine (AG) is an effective antioxidant and free radical scavenger which has long been known to protect against nephrotoxicity. We investigated the effects of AG on amikacin (AK)-induced changes of renal malondialdehyde (MDA), glutathione (GSH), blood urea nitrogen (BUN), serum creatinine (Cr) and albumin (Alb) which are used to monitor the development of renal tubular damage. Morphological changes in the kidney were also examined using light microscopy. A total of 21 rats were equally divided into three groups which were: (1) injected with saline, (2) injected with AK, and (3) injected with AK + AG, respectively. AK administration to control rats increased renal MDA and decreased GSH levels. AG administration before AK injection caused significant decreases in MDA and increases in GSH levels in kidneys compared to rats treated with AK alone. The serum BUN level increased slightly, Cr and serum Alb did not change as a result of any treatment. AG tended to decrease the level of serum BUN and did not cause any change in Alb or Cr levels. Morphological changes, including glomerular, tubular epithelial alterations and interstitial edema, were clearly observed in AK-treated rats. In addition, AG reversed the morphological damage to the kidney induced by AK. The results show that AG has a protective effect on nephrotoxicity induced by AK and may therefore improve the therapeutic index of AK.

Keywords Amikacin · Aminoguanidine · Malondialdehyde · Renal injury · Rat

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Introduction

Aminoglycoside antibiotics, including amikacin (AK), are commonly used for the treatment of severe gram negative bacterial infections. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic and ototoxic side effects [1]. Since the clinical use of aminoglycosides may be limited by the development of nephrotoxicity, it is important to know the risk factors that often involve a high incidence of renal damage. The nephrotoxic side effects of aminoglycoside antibiotics have been documented in numerous species of experimental animals [2, 3]. One mechanism of this toxicity is believed to be the generation reactive oxygen radical species (ROS); these agents likely account for the pathophysiology of aminoglycoside-induced nephrotoxicity [4, 5]. Aminoguanidine (AG), a compound structurally similar to L-arginine, inhibits inducible nitric oxide synthase (iNOS) in a selective and competitive manner, leading to the decreased generation of nitric oxide (NO) [6]. AG is endowed with many other activities that together account for its beneficial effects, such as inhibition of diamine oxidase [7] binding to sites of nonenzymatic glycosylation and preventing further advanced glycosylation [8]. It is significant that

previous studies have pointed to the beneficial antioxidant effects of AG [9] as well of peroxynitrite ($ONOO^-$) scavenger effects [10]. The protective effects of AG have been previously addressed in other models of cell damage induced by drugs [11]. The beneficial effects of AG in various experimental models of inflammation have also been reported [12]. Recently, Al-Shabanah et al. [13] showed that AG protects mice against hepatotoxicity induced by carbon tetrachloride, and Mansour et al. [14] reported that AG protects against nephrotoxicity induced by cisplatin (CDDP) in rats. The beneficial antioxidant effects of AG and the scavenger effects of $ONOO^-$, which is a reactive oxidant produced from NO and superoxide (O^-), have been observed in various forms of tissue injury.

Because iNOS production and oxyradical and $ONOO^-$ generation are the main cause of AK-induced renal injury, this experimental study was designed to investigate the possible protective effects of AG, a selective iNOS inhibitor and highly effective antioxidant and free radical scavenger, on nephrotoxicity induced by AK in a rat model, and to clarify the association between renal malondialdehyde (MDA), glutathione (GSH), blood urea nitrogen (BUN), serum creatinine (Cr) and albumin (Alb) levels and AK-induced nephrotoxicity.

Materials and methods

Experimental conditions

Female Wistar rats weighing 200–250 g were kept in a temperature (21 ± 2°C) and humidity (60 ± 5%) controlled room in which a 12:12 h light: dark cycle was maintained. Twenty-one rats were distributed equally into three groups: (1) injected with saline, (2) injected intraperitoneally (i.p.) with 1.2 g/kg AK (Amikozit 500 mg flk, Eczacıbası, Turkey) in a single dose, (3) injected i.p. in single dose of 1.2 g/kg AK plus 200 mg/kg AG 1 h before the AK for 3 days. At 24 h after the last injection, a time chosen according to our previous AK-related study [15], rats were killed and the kidneys quickly removed, decapsulated and divided equally into two longitudinal sections. One of these was placed in formaldehyde solution for routine histopathological examination by light microscopy. The other half was placed into liquid nitrogen and stored at –70°C until assayed for MDA, a lipid peroxidation product, and GSH levels. Trunk blood was extracted to determine the serum levels of BUN, Cr and Alb. For these studies, AG (Sigma-Aldrich Steinheim, Germany) was dissolved in saline (0.9% NaCl w/v) to obtain a final concentration of 200 mg/ml. Because of the very variable AG dosage schemes reported in literature, we administrated AG at the dose of 200 mg/kg/day i.p., which is reported to cause marked antioxidative and iNOS inhibitor effect [16].

All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Committee on Animal Research at Inonu University, Malatya.

Biochemical determination

A total of 200 mg of kidney tissue was homogenized in ice cold 150 mM KCl for the determination of MDA. The MDA content of the homogenates was determined spectrophotometrically by measuring the presence of thiobarbituric acid reactive substances [17]. GSH was determined by the spectrophotometric method, which was based on the use of Elman's reagent. [18]. Results are expressed as nmol/g tissue. Serum levels of BUN, Cr and Alb were determined using the Olympus Autoanalyser (Olympus Instruments, Tokyo, Japan).

Histological analysis

For light microscopic evaluation, portions of each kidney were fixed in 10% neutral phosphate buffered formalin solution. Following dehydration in an ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 6 μm were stained with hematoxylin-eosin (H-E) and examined using a light microscope (Olympus BH-2). Five coded slides from each group were examined by an observer blinded to treatment. The kidneys were examined for tubular alterations (vacuolization, cell desquamation and necrosis), interstitial edema, inflammatory cell infiltration and glomerular damage.

Statistical analysis

Kidney MDA, GSH, serum BUN, Cr and Alb levels were analyzed by one-way ANOVA. Post-hoc comparisons were done using Tukey's tests. Differences were considered significant at $P < 0.05$. Results are expressed as mean ± SEM.

Results

The effect of AG on AK-induced changes in lipid peroxides and GSH content

A single dose of AK-induced acute renal failure, manifested by a significant increase in kidney lipid peroxides measured as MDA while GSH content significantly decreased (Table 1). AG administration before AK injection caused significant decreases in MDA and increases in GSH levels in the kidney compared to in rats treated with AK alone.

Table 1 The effects of amikacin (AK) administration on rats with or without aminoguanidine (AG) treatment. [a] $P < 0.05$ vs control group, [b] $P < 0.05$ vs control + AK

Parameters	Control	Control + AK	Control + AK + AG
MDA (nmol/g tissue)	44.67 ± 2.60	147.09 ± 11.99[a]	73.83 ± 9.36[b]
GSH (nmol/g tissue)	245.56 ± 13.76	166.66 ± 15.06[a]	244.25 ± 14.46[b]
BUN (mg/dl)	19.16 ± 0.94	22.83 ± 3.15	24.16 ± 1.86
Cr (mg/dl)	0.46 ± 0.05	0.53 ± 0.08	0.53 ± 0.05
Alb (g/dl)	3.05 ± 0.11	3.11 ± 0.17	3.08 ± 0.04

The effect of AG on AK-induced changes in serum parameters

Serum levels of BUN were slightly, but not significantly, higher in the AK treated animals; Cr and Alb did not change significantly as a result of any treatment. Pre-treatment of the animals with AG tended to reduce the high level of serum BUN but did not change the levels of Cr or Alb.

Morphological damage ranged from none (control group; Fig. 1A) to moderate (AK + AG treated rats; Fig .1C), and severe (AK treated rats; Fig. 1B).

The control group revealed normal kidney parenchyma. The AK administrated group showed extreme cortical damage. Morphological changes including tubular epithelial alterations (vacuolization, atrophy and necrosis) and interstitial edema were clearly observed in the kidneys of the this group. Additionally to this changes, some of the glomeruli were damaged and glomerular capilleries were dilated and hypocellular. In the AK + AG administrated group, light microscope investigation revealed moderate degeneration and tubular necrosis as patchy areas in the renal cortex. Although interstitial edema was present in this group, the glomeruli were intact.

Discussion

Drugs with nephrotoxic potential are routinely used in prenatal and pediatric medicine, and an assessment of their relative toxicity is important. Aminoglycoside antibiotics have long been used in the antibacterial therapy of severe gram negative infections. [19]. Since the clinical use of aminoglycosides may be limited by the development of nephrotoxicity, it is important to be aware of those risk factors associated with a greater incidence of renal damage. A clear recognition of the patient—and treatment—related risk factors [20], combined with a once-a-day schedule and effective monitoring procedures [21], have definitely improved the situation over what prevailed in the early 1980s [22]. We are, however, still short of having advanced the safety of aminoglycosides to that of the other main wide spectrum antibiotics. Chemical research aimed at obtaining intrinsically less toxic compounds has met with only modest success, and few of the other approaches proposed to reduce the toxicities of the available agents have reached practical clinical applications. Yet, because aminoglycosides are very effective antibiotics well suited

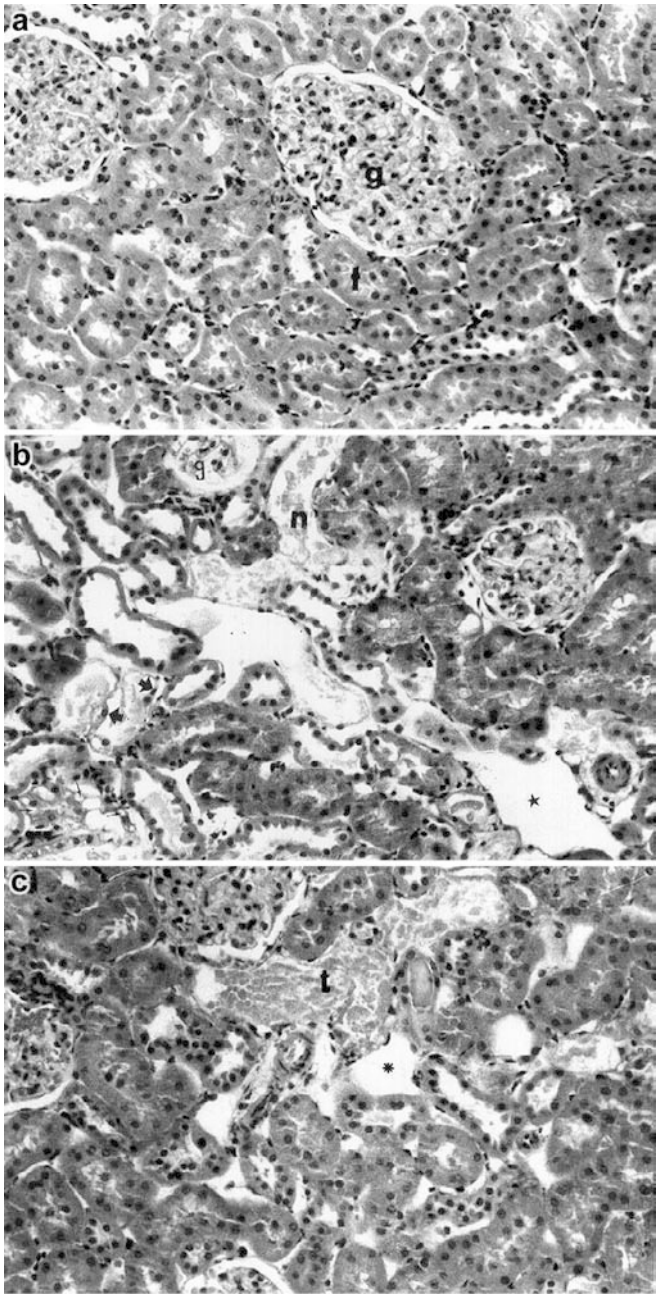

Fig. 1 **A** Control group; tubules (*t*) and glomeruli (*g*) appear normal. H-E ×66. **B** AK treated group; tubular vacuolization (*thin arrows*), epithelial cell atrophy (*thick arrows*) and necrosis (*n*) are seen. Notice that some of the glomeruli are damaged (*g*). Interstitial edema is evident (*). H-E ×66. **C** AK + AG group; glomeruli appear normal whereas moderate tubular injury (*t*) is present. Many of the tubules appear normal. Interstitial edema is still present (*). H-E ×66

to the treatment of severe infections [23], it seems important to maintain and even develop efforts to improve their therapeutic potential. Despite their beneficial effects, such as high antibacterial efficacy, rapid onset of action, low rate of true resistance, synergy with L-lactam antibiotics and low cost [1], aminoglycosides have considerable nephrotoxic side effects. Aminoglycoside toxicity may not only be due to others mechanism [24], but may also result from free radical damage, and antioxidant agents may reduce free radicals. AK is an aminoglycoside that is active against many strains of gram negative bacteria, as well as some other bacteria including some strains of staphylococci and mycobacteria. Nephrotoxicity is its chief adverse effect, and the monitoring of its plasma concentration is recommended. ROS have been implicated in a wide range of biological functions, but they can express both beneficial and highly toxic effects on cellular homeostasis [25]. Several conditions are known to disturb the balance between the production of ROS and cellular defences, resulting in dysfunction and cellular destruction. An imbalance between pro- and antioxidant factors plays an important role in many disease processes [26]. It has been also reported that ROS play a role in the pathophysiology of aminoglycoside induced nephrotoxicity [4, 5]. AG was prepared more than 100 years ago. During the last 10 years, two important effects have been discovered which have made this molecule attract a lot of interest. Firstly, AG inhibits, in vitro and in vivo, the formation of highly reactive advanced glycosylation end products (AGEs) [8] associated with the pathogenesis of secondary complications in diabetes and with cardiovascular changes in aging. AG ameliorates various complications in diabetes and prevents age related arterial stiffening and cardiac hypertrophy, effects probably dependent on the inhibition of AGEs formation. Secondly, AG inhibits NO synthase, particularly the inducible NO synthase isoform, making it an important pharmacological tool. The inducible NO synthase isoform is associated with the production of large quantities of NO synthase in response to, e.g., cytokines. AG is endowed with many other activities that together account for its beneficial effects. It inhibits diamine oxidase [7] which catalyzes the degradation of biologically active diamines such as histamine and putrescine. It is significant that previous studies have pointed to the beneficial antioxidant effects [9] and $ONOO^-$ scavenger effects [10] of AG. Yildiz et al. [27] also found that AG has direct scavenging activities against hydroxyl radicals. Recently, Giardino et al. [28] reported that AG acted as an antioxidant in vivo, preventing ROS formation and lipid peroxidation in cells and tissues, preventing oxidant-induced apoptosis. It has been proposed that antioxidants maintain the concentration of reduced GSH and may restore the cellular defense mechanisms and block lipid peroxidation, thus protecting against the toxicity for wide variety of nephrotoxic chemicals [29]. Depletion of renal glutathione (GSH), which is one the primary reasons for the resulting lipid peroxidation and the increase in MDA, an end product of lipid peroxidation, have been observed in CDDP treated rats [30]. We also showed [18] that antioxidants reduced ischemia-reperfusion induced MDA production in the kidney. As with the current findings, these results indicate that the generation of free radicals and subsequent lipid peroxidation may play a role in AK toxicity.

Because both ROS formation and iNOS generation are two main causal factors related to AK-induced renal injury, we used the potent antioxidant and iNOS inhibitor AG in the current study. We determined the effects of AG on AK-induced changes in renal MDA, GSH, BUN, Cr and Alb. Morphological changes in the kidney were also examined using light microscopy. We documented renal MDA and GSH levels. AK administration induced increased renal MDA and decreased GSH levels. Administration of AG before AK injection caused a significant decreases in lipid peroxidation and increases in GSH levels in the kidney compared to rats treated with AK alone. These findings may indicate an improvement in the oxidant status and the possible antioxidant activity of AG. In addition, they strongly suggest that AG is important in protecting the kidney from AK-induced injury. Serum levels of BUN were slightly, but not significantly, higher in the AK treated animals, whereas Cr and Alb did not change as a result of any treatment. These results parallel those of our previous study [30], which investigated CDDP-induced renal injury. Pre-treatment of the animals with AG tended to reduce the high level of serum BUN and did not change the levels of Cr and Alb. Recent studies tested the beneficial effects of AG in the prevention of diabetic complications in several tissues, including the kidney. The treatment of rats with AG alone (100 mg/kg per day p.o.) for 10 days did not induce any change in the measured biochemical parameters [31]. On the other hand, the results of Mansour et al. [14] indicate that the administration of AG in drinking water (100 mg/kg p.o.) for 5 days consecutively before and continued for another 5 consecutive days after a single dose of CDDP (7.5 mg/kg i.p.) renders rats less susceptible to kidney damage induced by treatment with CDDP. This protection was evidenced in the serum as an elevated level in BUN, but Alb and Cr tended to be normal. The attenuation of the nephrotoxicity was also observed in the kidney. These results confirm previous data demonstrating the beneficial antioxidant effect of AG [9]. In our study, AG afforded substantial protection against nephrotoxicity induced by AK. Our biochemical results correlated well with the renal histological examination which revealed that the AK administrated group showed extreme cortical damage, tubular epithelial alterations, interstitial edema and damaged glomerular structure. The administration of AG reversed the damage induced by AK.

AG replacement therapy or AG supplementation may reduce AK-induced renal injury. We propose that AG acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of AK both at the

biochemical and histological levels. Thus, we believe that it could be effectively combined with AK. Further studies are needed to elucidate the mechanisms of protection and the effect of AG.

References

1. Begg EJ, Barclay ML (1995) Aminoglycosides-50 years on. Br J Clin Pharmacol 39: 597
2. Klein J, Koren G, McLeod SM (1992) Comparison methods for prediction of nephrotoxicity during development. Dev Pharmacol Ther 19: 80
3. Ibrahim S, Langhendries JP, Bernard A, Tulkens PM (1994) Urinary phospholipids excretion in neonates treated with amikacin. Int J Clin Pharmacol Res 14: 149
4. Baliga R, Zhang Z, Baliga M, Ueda N, Shah SV (1998) In vitro and in vivo evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. Kidney Int 53: 394
5. Rao M, Kumar MM, Rao MA (1999) In vitro and in vivo effects of phenolic antioxidants against cisplatin-induced nephrotoxicity. J Biochem 125: 383
6. Misko TP, Moore WM, Kasten TP, Nickols GA, Corbett JA, Tilton RG, McDaniel ML, Williamson JR, Currie MG (1993) Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. Eur J Pharmacol 233: 119
7. Tilton RG, Chang K, Hasan KS, Smith SR, Petrash JM, Misko TP, Moore WM, Currie MG, Corbett JA, McDaniel ML (1993) Prevention of diabetic vascular dysfunction by guanidines. Inhibition of nitric oxide synthase versus advanced glycation endproduct formation. Diabetes 42: 221
8. Sugimoto K, Yagihashi S (1997) Effects of aminoguanidine on structural alterations of microvessels in peripheral nerve of streptozotocin diabetic rats. Microvasc Res 53: 105
9. Ihm SH, Yoo HJ, Park SW, Ihm J (1999) Effect of aminoguanidine on lipid peroxidation in streptozotocin induced diabetic rats. Metabolism 48: 1141
10. Szabo C, Ferrer-Sueta G, Zingarelli B, Southan GJ, Salzman AL, Radi R (1997) Mercaptoethylguanidine and guanidine inhibitors of nitric-oxide synthase react with peroxynitrite and protect against peroxynitrite-induced oxidative damage. J Biol Chem 272: 9030
11. Aoki K, Ohmori M, Takimoto M, Ota H, Yoshida T (1997) Cocaine-induced liver injury in mice is mediated by nitric oxide and reactive oxygen species. Eur J Pharmacol 336: 43
12. Shiomi M, Wakabayashi Y, Sano T, Sinoda Y, Nimura Y, Ishimura Y, Suematsu M (1998) Nitric oxide suppression reversibly attenuates mitochondrial dysfunction and cholestasis in endotoxemic rat liver. Hepatology 27: 108
13. Al-Shabanah OA, Alam KS, Nagi MN, Al-Rikabi AC, Al-Bekairi AM (2000) Protective effect of aminoguanidine, a nitric oxide synthase inhibitor, against carbon tetrachloride induced hepatotoxicity in mice. Life Sci 66: 265
14. Mansour MA, Mostafa AM, Nagi MN, Khattab MM, Al-Shabnah OA (2002) Protective effect of aminoguanidine against nephrotoxicity induced by cisplatin in normal rats. Comp Biochem Physiol C 132: 123
15. Parlakpinar H, Ozer MK, Sahna E, Vardi N, Cigremis Y, Acet A (2003) Amikacin-induced acute renal injury in rats protective role of melatonin. J Pineal Res 35: 85
16. Takizawa S, Fukuyama N, Hirabayashi H, Nakazawa H, Shinohara Y (1999) Dynamics of nitrotyrosine formation and decay in rat brain during focal ischemia-reperfusion J Cereb Blood Flow Metab 19: 667
17. Vermeulen NPE, Baldew GS (1992) The role of lipid peroxidation in the nephrotoxicity of cisplatin. Biochem Pharmacol 44: 1193
18. Sahna E, Parlakpinar H, Ozturk F, Cigremis Y, Acet A (2003) The protective effects of physiological and pharmacological concentrations of melatonin on renal ischemia-reperfusion injury in rats. Urol Res 31: 188
19. Walker RJ, Duggin GG (1988) Drug nephrotoxicity. Annu Rev Pharmacol Toxicol 28: 331
20. Moore RD, Lietman PS, Smith C (1987). Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. J Infect Dis 155: 93
21. Prins JM, Weverling GJ, De Blok K, Van Ketel RJ, Speelman P (1996) Validation and nephrotoxicity of a simplified once-daily aminoglycoside schedule and guidelines for monitoring therapy. Antimicrob Agents Chemother 40: 2494
22. Smith CR, Lipsky JJ, Laskin OL, Hellman DB, Mellits ED, Longstreth J, Lietman PS (1980) Double-blind comparison of the nephrotoxicity and auditory toxicity of gentamicin and tobramycin. N Engl J Med 302: 1106
23. Gilbert DN (1995) Aminoglycosides. Principles and practice of infectious diseases, 4th edn. Churchill Livingstone, New York, p 279
24. Hagiwara M, Inagaki M, Kanamura K, Ohta H, Hidaka H (1988) Inhibitory effects of aminoglycosides on renal protein phosphorylation by protein kinase C. J Pharmacol Exp Ther 244: 355
25. Halliwell B, Gutteridge JMC, Cross CE (1992) Free radicals, antioxidants and human disease. Where are we now? J Lab Clin Med 119: 598
26. Wolff SP (1993) Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stressing the etiology of diabetes mellitus and complications. Br Med Bull 49: 642
27. Yildiz G, Demiryurek AT, Sahin-Erdemli I, Kanzik I (1998) Comparison of antioxidant activities of aminoguanidine, methyl-guanidine and guanidine by luminol-enhanced chemiluminescence. Br J Pharmacol 124: 905
28. Giardino I, Fard AK, Hatchell DL, Brownlee M (1998) Aminoguanidine inhibits reactive oxygen species formation, lipid peroxidation, and oxidant-induced apoptosis. Diabetes 47: 1114
29. Babu E, Gopalakrishnan VK, Sriganth IN, Gopalakrishnan R, Sakthisekaran D (1995) Cisplatin induced nephrotoxicity and the modulating effect of glutathione ester. Mol Cell Biochem 144: 7
30. Parlakpinar H, Sahna E, Ozer MK, Ozugurlu F, Vardi N, Acet A (2002) Physiological and pharmacological concentrations of melatonin protect against cisplatin induced acute renal injury. J Pineal Res 33: 161
31. Sugimoto H, Shikata K, Wada J, Horiuchi S, Makino H (1999) Advanced glycation end products–cytokine–nitric oxide sequence pathway in the development of diabetic nephropathy: aminoguanidine ameliorates the overexpression of tumor necrosis factor-alpha and inducible nitric oxide synthase in diabetic rat glomeruli. Diabetologia 42: 878