Effect of Panax ginseng on gentamicin sulphate-induced kidney toxicity in rats

A. KARADENIZ1, A. YILDIRIM2, N. SIMSEK2, H. TURHAN2, Y. KALKAN4, F. CELEBI4

1 Department of Physiology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, TURKEY.
2 Department of Histology and Embryology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, TURKEY.
3 Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum, TURKEY.
4 Department of Histology and Embryology, Institute of Health Sciences, Atatürk University, Erzurum, TURKEY.

SUMMARY

The protective effects of Panax ginseng (PG) on gentamicin sulphate (GS) induced nephrotoxicity were investigated in rats. A total of 32 adult Sprague Dawley rats were randomly divided into 4 equal groups and treated by intraperitoneal route for 10 days with: 0.5 mL of isotonic saline (group C), GS 100 mg / kg / day (group GS), PG 200 mg / kg / day (group PG) and GS (100 mg / kg / day) plus PG (200 mg / kg / day) (group PG + GS). After the last injection, plasma urea and creatinine concentrations and kidney MDA (malondialdehyde), NO (nitric oxide) and GSH (glutathione) concentrations as well as tissue GPX (glutathione peroxidase) and SOD (superoxide dismutase) activities were measured by spectrophotometry. In parallel, kidney injury was histopathologically evaluated. Marked elevations of plasma urea and creatinine concentrations and severe tubular necroses in all rats (except one) confirmed the GS toxicity. Besides, treatment with GS alone induced significant increases of tissue oxidant (MDA and NO) concentrations associated to impairment of antioxidant systems (decreases of GSH concentrations and of GPX and SOD activities). By contrast, co treatment with PG significantly alleviated the GS-induced oxidative stress (decreases of MDA and NO contents and heavy restoration of antioxidants) and has partially protected rats from nephotoxicity (reduction of kidney damage and of plasma urea and creatinine concentrations). These results indicate that the nephroprotective effects of Panax ginseng against GS-induced oxidative damage may be due to its antioxidant and free radical scavenging activities.

Key-words: Panax ginseng, gentamicin sulphate, nephrotoxicity, oxidative damage.

RÉSUMÉ

Effet du ginseng du Panax sur la toxicité rénale de la gentamycine chez le rat

Les effets protecteurs du ginseng du Panax (PG) sur la néphrotoxicité induite par le sulfate de gentamycine (GS) ont été étudiés chez le rat. Trente deux rats adultes Sprague Dawley ont été aléatoirement répartis en 4 groupes égaux et traités par voie intrapéritonéale pendant 10 jours avec: 0.5 mL de sérum physiologique (groupe C), le sulfate de gentamycine (100 mg / kg / j) (groupe GS), le ginseng du panax (200 mg / kg / j) (groupe PG) ou, à la fois le GS (100 mg / kg / j) et le PG (200 mg / kg / j) (groupe PG + GS). À l’issue de la dernière injection, les concentrations plasmatiques et tissulaires en MDA (malondialdehyde), NO (oxyde nitrique), GSH (glutation) et les activités tissulaires de la GPX (glutation peroxydase) et de la SOD (superoxide dismutase) ont été déterminées par spectrophotométrie. En parallèle, une analyse histopatologique a été réalisée sur les reins. La toxicité du GS a été confirmée par les fortes élévations de l’urémie et de la créatininémie et par le développement d’une nécrose tubulaire sévère chez tous les rats (sauf un). De plus, le traitement par le GS seul a induit des augmentations significatives des concentrations tissulaires en oxydants (MDA et NO) associées aux déficits des systèmes anti-oxydants (diminutions de la concentration tissulaire de GSH et des activités GPX et SOD). En revanche, le co-traitement par le PG a significativement atténué les phénomènes d’oxydation liés au GS (réduction des teneurs tissulaires en MDA et en NO et restauration des systèmes anti-oxydants) et a protégé ainsi, au moins partiellement, les rats de la toxicité du GS (atténuation des lésions rénales et réduction des variations de l’urémie et de la créatininémie). Ces résultats indiquent que la néphrotoxicité du GS est liée à l’apparition d’un stress oxydatif et que les effets néphroprotecteurs du PG seraient dus à son caractère anti-oxydant (par neutralisation des radicaux libres).

Mots-clés : Ginseng du Panax, sulfate de gentamycine, néphrotoxicité, lésions oxydatives.

Introduction

The clinical use of aminoglycosides may be limited by the risk of nephrotoxicity. The most widely used aminoglycoside is the gentamicin sulphate (GS) [15], but the GS nephrotoxicity is responsible for 10–20% of all cases of acute renal failure according to experimental results [3]. GS-induced nephrotoxicity is characterized by direct tubular necrosis without morphological changes in glomerular structures [11, 12, 30] and results from the drug accumulation in the proximal convoluted tubules, leading to the loss of the brush border integrity [7]. Although the pathogenesis is still not well understood, the toxicity of GS in the kidney seems to be related to the generation of destructive reactive oxygen species (ROS) in these cells [4, 37].

ROS have been proposed as causative agents of cell death in many different pathological states as well as in glomerular diseases [42], in renal ischemia and reperfusion injury [19], and in various models of toxic renal failure [32]. RAMA-SAMMY et al. [35, 36] have demonstrated that renal cortical lipid peroxidation increased in GS-treated rats and that hydrogen peroxide (H2O2) was intensively produced in the
cortical mitochondria in vitro [47]. Some investigators also showed that GS acts as an iron chelator and that the iron-GS complex is a potent catalyst of free radical formation [35, 51].

Several agents such as lipoic acid, S-allylmercaptocysteine, Diallyl disulfide, L-arginine, carvedilol, fish oil, garlic extract, carotenoids, vitamin E and C [1, 2, 20, 21, 30, 31] have been tested for alleviating GS nephrotoxicity. As the constituents of Panax ginseng (PG) are scavengers of free radicals, PG inhibits lipid peroxidation, contributes to the maintain of the integrity and permeability of cell membranes and protects cells and tissues against oxidative stress induced by free radicals. Its antioxidant activity is attributed to its capacity to increase expression of free radical-scavenging enzymes [41]. Consequently, PG would exert beneficial effects against renal damage and the aim of this study was to investigate the potential protective effect of PG on renal damage induced by GS in rats.

Material and Methods

1. CHEMICALS

Gentamicin sulphate (GS) and Panax ginseng (PG) were purchased from Vetas (Istanbul, Turkey) and trade cooperation Saglık ve Güzellik Merkezi (SGM) (Ankara, Turkey) respectively. All other chemicals of analytical grade were purchased from Sigma Chemical Co. (St. Louis, MO) or Merck (Darmstadt, Germany).

2. ANIMALS AND EXPERIMENTAL DESIGN

In this study, 32 healthy adult male Sprague-Dawley rats weighting between 200-250 g were used. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (24 ± 3°C) during the experimental period. All experimental procedures were conducted in accordance with the guide to the care and use of laboratory animals (Atatürk University, Experimental Research Centre, Erzurum, Turkey). Water and food (standard commercial rat diet) were provided ad libitum. The animals were randomly divided into four equal groups (n = 8). In the control group (group C), rats received a daily intraperitoneal (i.p.) injection of 0.5 ml isotonic saline for 10 days. In the PG and GS groups, rats were intraperitoneously daily treated by PG (200 mg / kg / day) and by GS (100 mg / kg / day) respectively for 10 days, whereas in the PG + GS group, animals were simultaneously injected by the 2 drugs (PG: 200 mg / kg / day and GS: 100 mg / kg / day) for 10 days.

3. SAMPLE COLLECTION AND BIOCHEMICAL ASSAYS

All rats were anesthetized with ketamine (50 mg / kg) and xylazine (5 mg / kg) intramuscularly, then killed by cervical dislocation 24 h after the last injection and trunk blood was collected into heparinized tubes and centrifuged (1 500 g for 10 minutes at room temperature (25°C). Plasma samples and the kidneys were collected and stored -70°C for biochemical analysis.
All tissues were maintained at +4°C throughout the different steps of preparation. Portions of each kidney tissue (1.9 w/v) were homogenized in 0.9 % NaCl solution with an OMNI TH International homogenizer (Warrenton, VA, USA). Tissue homogenates were centrifuged for 15 min at 15,000 g at 4°C and then the clear upper supernatants were removed for analyses.

The MDA concentrations were determined according to the method of OHKAWA et al. [25] and were expressed as nmol / g of proteins. The tissue GSH concentrations were measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by TITZETE [46] and ANDERSON [5], and were expressed as µmol / g protein. The GPX and SOD activities were determined by the procedure described by BEUTLER [8] and by the method of SUN et al. [44] respectively, and were expressed as U / g protein. The NO concentrations were measured using the Griess reagent by the method of MOSHAGE et al. [22], and were expressed as µmol / g protein. The plasma creatinine and urea concentrations were measured using an auto analyzer with corresponding commercial kits (Roche, Mannheim, Germany) and were expressed as g or mg / L.

4. HISTOPATHOLOGICAL EXAMINATIONS

The second kidney was excised after cervical dislocation and was fixed in 10 % formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin-eosin. Light microscopy was used to evaluate tubular necrosis, which was graded as follows: mild (+), only single cell necrosis and slight degenerative changes; moderate (++), tubular necrosis at different foci throughout the cortex; severe (+++), extensive and marked tubular necrosis throughout the cortex.

5. STATISTICAL ANALYSIS

The data were expressed as means ± standard errors (S.E.M.). Differences between group means were estimated using a one-way analysis of variance followed by Tukey’s test using the SPSS 12.0 for Windows. Results were considered statistically significant at p < 0.05.

Results

1. EFFECTS OF GS AND PG TREATMENTS ON PLASMA BIOCHEMICAL PARAMETERS

Plasma urea and creatinine concentrations were markedly increased in the GS group compared to controls (p < 0.05). Although treatment with PG alone has also induced a significant increase of the urea concentrations compared to control (p < 0.05), the combined treatment (PG + GS) has not dramatically induced elevations of the plasma biochemical parameters: values observed in the PG + GS group were significantly higher than control values (p < 0.05) but they were significantly reduced compared to the GS group (p < 0.05).

2. EFFECTS OF GS AND PG TREATMENTS ON THE KIDNEY OXIDANT / ANTIOXIDANT BALANCE

The effects of the different treatments on the kidney oxidant / antioxidant balance were presented in Table 1. Treatment with GS alone has dramatically enhanced the kidney contents of oxidants (MDA and NO) compared to the controls (p < 0.05) whereas tissue GSH concentrations and antioxidant enzyme (SOD and GPX) activities were significantly depressed (p < 0.05).

By contrast, in the PG group, MDA contents were significantly reduced compared to controls (p < 0.05) whereas the GSH contents and the GPX activity were enhanced (p < 0.05). The treatment with PG alone has not significantly affected the NO concentrations either the SOD activity. Nevertheless, when rats were submitted to combined treatment (PG + GS group), the kidney MDA and NO oxidant concentrations were intensively lowered compared to values recorded in the GS group (p < 0.05) or in the control group (p < 0.05) and the antioxidant capacity was markedly restored: tissue GSH concentrations and SOD activities were maximal in this group (p < 0.05 compared to controls and to the 2 other assay groups). Surprisingly, even if GPX activity was higher in the PG + GS group than in the GS group (p < 0.05), the enzyme activities remained significantly lowered compared to the observed activities in the PG and the control groups (p < 0.05).

3. HISTOPATHOLOGICAL ANALYSIS

The histological changes were evaluated and presented in Table II. The kidneys of animals from the control and the PG treated groups showed normal histology (Figure 1). Treatment with GS alone caused a severe proximal tubular necrosis (+++), desquamation and parenchyma degeneration (Figure 2) in all rats except for one in which only moderate lesions were recorded. Co-treatment with PG decreased the tubular necrosis intensity and caused ameliorative changes (Figure 3): 5 rats exhibited mild (+) lesions and three moderate (2+) lesions. Slight desquamation and relative atrophy of the tubular epithelial cells were observed in this group.

Discussion

Nephrotoxicity is a major complication of the GS administration. Therefore, the reduction of nephrotoxicity would enhance its clinical value. Several antioxidants that scavenge or interfere with the production of ROS have been used successfully to ameliorate GS nephropathy [2, 7]. In the present study we focused on the effects of PG on the renal damage and oxidative injury induced by GS.

In this study, it was shown that treatment with only GS caused nephrotoxicity in rats, evidenced by high plasma urea and creatinine concentrations and by histological lesions (severe tubular necrosis). Similar structural changes were also reported by KUMAR et al. [18] and NAKAKUKI et al. [24]. Plasma creatinine concentration is a more potent indicator...
GPX (U/L) 11.03 ± 0.40 c 13.21 ± 0.23 d 8.07 ± 0.44 a 9.85 ± 0.28 b

GSH

CAT, SOD and low GSH content in the renal cortex. Nephropathy was associated with low activities of GPX, the increase of its concentrations in the kidney tissue revealed to oxidation of polyunsaturated fatty acids by free radicals, results were also observed by OZBEK [26]. The decreased renal antioxidant capacity due to excessive ROS production could aggravate oxidative damage in rats. GSH is an essential compound for maintaining cellular integrity and metabolism. However, the effects of xenobiotics or peroxide-dependent alterations on the tissue GSH contents and the antioxidant enzyme activities are currently debated. It has been proposed that antioxidants maintain the concentration of reduced GSH, restore the antioxidant mechanisms and block lipid peroxidation, preventing in this way the toxicity of various nephrotoxic chemicals [6]. Some investigators [28, 30, 48] suggested that GS caused the depletion of kidney GSH concentrations, leading to impairment of antioxidant systems and to exacerbated lipid peroxidation. In agreement with that, kidney GSH concentrations and GPX/SOD activi-

Creatinine (mg/L) 6.2 ± 0.8 a 6.6 ± 1.0 a 14.2 ± 2.1 c 8.9 ± 1.3 b

Urea (g/L) 0.47 ± 0.04 a 0.65 ± 0.07 b 1.05 ± 0.07 c 0.70 ± 0.06 b

**Plasma**

**Kidney**

MDA (nmol/g) 73.40 ± 5.20 c 58.60 ± 1.21 a 121.60 ± 6.12 d 63.60 ± 3.67 b

NO (μmol/g) 100.56 ± 1.45 b 104.79 ± 3.39 b 156.09 ± 8.61 c 68.93 ± 6.10 a

GSH (μmol/g) 11.27 ± 0.53 b 13.30 ± 1.15 c 10.03 ± 0.27 a 14.84 ± 0.62 c

GPX (U/L) 11.03 ± 0.40 c 13.21 ± 0.23 d 8.07 ± 0.44 a 9.85 ± 0.28 b

SOD (U/L) 1.58 ± 0.07 b 1.61 ± 0.09 b 1.28 ± 0.14 a 1.91 ± 0.11 c

Different superscripts a, b, c, d in the same line indicate significant differences between groups (p < 0.05).

**TABLE 1:** Variations of the plasma urea and creatinine concentrations and of the kidney oxidants (MDA and NO) and antioxidants (GSH and SOD / GPX activities) according to the treatments of rats (n = 8 in each group): group C (controls), group PG (panax ginseng: 200 mg / kg / day, i.p., for 10 days), group GS (gentamicin sulphate: 100 mg / kg / day, i.p., for 10 days) and group PG + GS (panax ginseng: 200 mg / kg / day and gentamicin sulphate: 100 mg / kg / day, i.p., for 10 days). Results are expressed as mean ± SEM.

Tubular necrosis was graded as follows: severe necrosis (+++): extensive and marked necrosis through the cortex, moderate necrosis (++): necrosis at different foci through the cortex, mild necrosis (+): slight degenerative changes, absence of necrosis (-).

**TABLE 2:** Number of rats with histological damage according to the treatment (n = 8 in each group): group C (controls), group PG (panax ginseng: 200 mg / kg / day, i.p., for 10 days), group GS (gentamicin sulphate: 100 mg / kg / day, i.p., for 10 days) and group PG + GS (panax ginseng: 200 mg / kg / day and gentamicin sulphate: 100 mg / kg / day, i.p., for 10 days).

The exact mechanisms by which GS induced nephrotoxicity are not well clarified. The interaction between cationic drugs such as aminoglycosides and the anionic phospholipids is considered as the first step for the development of GS toxicity [50]. But, some authors [7, 33, 51] reported that GS and some antibiotics cause iron release from renal cortical mitochondria and acts as iron chelators, the formed iron-drug complexes being potent catalysts of free-radical formation. Several researchers reported that aminoglycosides are able to cause the formation of ROS, particularly of H2O2 and O2.− [7, 48], which are directly involved in GS-induced damage [27, 49] by inducing mesangial cells contraction [39], altering the filtration surface area and modifying the ultrafiltration coefficient, these modifications leading to the decrease of the glomerular filtration rate. In this way, GS treatment has induced a strong accumulation of oxidants (MDA and NO) in the kidney while SOD and GPX enzyme activities and GSH contents were significantly decreased in the present study. Similar results were also observed by OZBEK et al. [26]. GS nephropathy was associated with low activities of GPX, CAT, SOD and low GSH content in the renal cortex.

Because MDA is the end product of lipid peroxidation due to oxidation of polyunsaturated fatty acids by free radicals, the increase of its concentrations in the kidney tissue revealed an enhanced lipid peroxidation leading to tissue damage as well as the failure of the antioxidants to prevent excessive production of free radicals. Although NO is stable in low concentrations and in absence of oxygen, it becomes highly reactive in the presence of biological environment where oxygen and free radicals exist [16, 41]. In the present study, the kidney NO concentrations were significantly higher in the GS treated group than in the control group. This indicates that aminoglycoside-induced renal injury is related to, or at least partially mediated by, the NO generation and the subsequent formation of other free radicals. In agreement with that, it is recently reported that aminoguanidine, a specific iNOS inhibitor and especially a NO3 scavenger, can have protective effects on amikacin-induced renal injury [28].

ties were significantly lowered compared to controls in the present study. Nevertheless, other authors [10, 17] have reported that GS exposure stimulated endogenous defences evidenced by increases of antioxidant enzyme activities and tissue glutathione concentrations. In rats treated with GS, the decrease of GPX activity could be associated with the necrosis of proximal tubules, the primary site of drug accumulation, because this enzyme is synthesized almost exclusively in proximal tubular cells [29]. Moreover, the observed decreases of SOD activity would specifically promote the accumulation of O$_2^-$ in kidney.

Several studies have reported favourable effects of free radical scavengers in various models of kidney injury [23, 48]. In the case of GS, various agents can partially prevent the GS-induced renal damage: for example, treatment with the polyaspartic acid prevented functional and histological changes of GS-induced nephrotoxicity [14]. The administration of L-arginine [9], melatonin [40], carvedilol [18], some medicinal plants such as arabic gum [1, 2, 4] and oils (fish oil and sunflower oil) [1, 2] successfully alleviated the GS-induced nephropathy. In another study, pretreatment with superoxide dismutase (8000 IU / kg) offered a significant protection against GS-induced nephrotoxicity [3]. Because of the well-known antioxidative / anti-inflammatory [38, 41, 43, 45] and antiproliferative [34, 52] properties of GS, this experimental study was planned to investigate its potential protective effects on GS-induced renal changes. In the present study, the increases of plasma creatinine and urea concentrations induced by GS were considerably limited by cotreatment with PG. In parallel, the gravity of histological lesions was significantly attenuated: only mild or moderate tubular necroses were still observed in rats receiving simultaneously GS and PG.

Several clinical studies and in vitro / in vivo experiments [38, 41, 52] have already demonstrated the beneficial effects of PG. It is stated that the phenolic acids, flavonoids and saponins in ginseng are responsible for its antioxidant properties [43] by scavenging and destroying free radicals and ROS (such as O$_2^-$). Indeed, in the present study, treatment with the polyaspartic acid prevented functional and histological changes of GS-induced nephrotoxicity [14]. The administration of L-arginine [9], melatonin [40], carvedilol [18], some medicinal plants such as arabic gum [1, 2, 4] and oils (fish oil and sunflower oil) [1, 2] successfully alleviated the GS-induced nephropathy. In another study, pretreatment with superoxide dismutase (8000 IU / kg) offered a significant protection against GS-induced nephrotoxicity [3]. Because of the well-known antioxidative / anti-inflammatory [38, 41, 43, 45] and antiproliferative [34, 52] properties of GS, this experimental study was planned to investigate its potential protective effects on GS-induced renal changes. In the present study, the increases of plasma creatinine and urea concentrations induced by GS were considerably limited by cotreatment with PG. In parallel, the gravity of histological lesions was significantly attenuated: only mild or moderate tubular necroses were still observed in rats receiving simultaneously GS and PG.

Several clinical studies and in vitro / in vivo experiments [38, 41, 52] have already demonstrated the beneficial effects of PG. It is stated that the phenolic acids, flavonoids and saponins in ginseng are responsible for its antioxidant properties [43] by scavenging and destroying free radicals and ROS (such as O$_2^-$). Indeed, in the present study, treatment with PG alone has significantly reduced renal MDA concentrations and improved GSH contents as well as the GPX activity compared to controls. Furthermore, co-treatment with PG has significantly prevented the GS-induced lipid peroxidation in kidney tissue: compared to the GS group, tissue MDA and NO concentrations were markedly depressed whereas the activities of antioxidant enzymes (SOD and GPX) and GSH concentrations were significantly enhanced. The antioxidants found in PG have considerably limited the accumulation of free radicals and ROS, and the NO reactivity in an oxidant environment may be strongly diminished. Consequently, the lipid peroxidation and cellular damage were dramatically alleviated. Similar antioxidant properties have also been reported in studies using ischemia re-perfusion injury rat brain [19]. Moreover, cellular GSH pool was no more intensively consumed, leading directly and indirectly to the restoration of the activity of the antioxidant enzymes, SOD and GPX at a lesser extend. Nevertheless, as the PG dosage was not sufficient for completely counteracting the deleterious effects of GS, necrosis of proximal tubules was still observed and some GPX proteins which are specifically expressed by the proximal tubules were lost. In this way, this enzyme may be considered as a marker of tubular damage extent.

In conclusion, the GS-induced nephrotoxicity may be related to oxidative damage. Co-administration of PG decreased the harmful effects of GS by inhibiting free radical formation and by restoration of the antioxidant systems. Further investigations on the mechanism of action of PG are required and may have a considerable impact on future clinical treatments of patients with renal failure.

References


