Protective Effect of Quercetin against Gentamicin-Induced Nephrotoxicity in Rats

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Gentamicin (GM) is an antibiotic widely used in treating severe gram-negative infections. However, its clinical use is limited by its nephrotoxicity. Several lines of evidence indicate that free radicals are important mediators of gentamicin nephrotoxicity. Therefore, the aim of this work was to investigate the possible protective effect of the flavonoid quercetin, an antioxidant, on gentamicin-induced nephrotoxicity. For this purpose, rats were divided into four groups. First group served as a control and injected with the normal saline, second group was injected with quercetin (50 mg/kg/d, per os) for 7 d, third group was injected with gentamicin (80 mg/kg/d, intraperitoneally) for 7 d and the fourth group of animals was injected with quercetin plus gentamicin simultaneously for 7 d. Total protein levels were estimated in 24-h urine samples to assess kidney dysfunction. The rats were sacrificed on the seventh day and kidneys were collected for histopathological studies. Blood urea nitrogen (BUN) and creatinine levels were measured in the blood. Moreover, glutathione (GSH), lipid peroxide (TBARS) levels, superoxide dismutase (SOD) and catalase (CAT) activities were determined in renal tissues. GM-treated rats showed early kidney dysfunction as urinary total protein, BUN and serum creatinine levels were significantly increased. The significant decrease in GSH levels, SOD, CAT activities and increase in TBARS levels, indicated that GM-induced nephrotoxicity was mediated through oxidative stress reactions. Histopathological examination of GM-treated rats revealed degenerative changes in glomeruli and tubules. On the other hand, simultaneous administration of quercetin plus gentamicin protected kidney tissues against nephrotoxic effects of gentamicin as evidenced from amelioration of histopathological changes and normalization of kidney biochemical parameters.

Key words quercetin; gentamicin; nephrotoxicity; antioxidant; oxidative stress reaction

Gentamicin (GM) is an aminoglycoside antibiotic that is very effective in treating life-threatening gram-negative infections.1) Unfortunately, 30% of patients treated with GM for more than 7 d show some signs of nephrotoxicity.2–3) It has been reported that GM-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule.4) The specificity of gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules (50 to 100 times greater than serum).4) The exact mechanism of GM-induced nephrotoxicity is unknown. However, GM has been shown to enhance the generation of reactive oxygen species (ROS)5)–7) causing deficiency in intrinsic antioxidant enzymes.8) ROS have been suggested as a cause of death for many cells in different pathological states including various models of renal and cardiac diseases.9–11) Accordingly, the use of several compounds with antioxidant activity has been successfully used to prevent or ameliorate GM-induced nephrotoxicity.7) Flavonoids comprise a large group of secondary metabolites occurring widely throughout the plant kingdom including food plants.12) The daily flavonoid intake (mainly from onions, apples, grapes, wine, tea, berries, herbs and spices) in the human diet is highly variable. It is ranged from 23 mg/d (only flavonol plus flavones)13) to more than 500 mg/d (total flavonoids).14) Among dietary flavonoids, quercetin is by far the most abundant. Quercetin, (3,5,7,3’,4’-pentahydroxy flavone)15) is a natural polyphenolic flavonoid widely found in edible plants (i.e. fruits, vegetables, herbs, grains) and beverages (i.e. tea, red wine).16) It has been reported that quercetin inhibited thrombocyte aggregation16) and had an anti-hypertensive effect.17) Interest in dietary phenolics has increased greatly recently, owing to their antioxidant properties (free radical scavenging and metal chelating) and their possible beneficial implications in human health, such as in the treatment and prevention of cancer and cardiovascular diseases.18) Quercetin has already been shown to reduce the oxidative stress in streptozotocin-induced diabetic rats.19,20) However, until now, the protective effect of quercetin against GM-induced nephrotoxicity has not been investigated.

Based on the above mentioned data, the hypothesis was made that quercetin could ameliorate GM-induced oxidative stress and renal damage. Therefore, the major objective of this study was to investigate the possible protective effect of quercetin against GM-induced renal damage, which was evaluated by measuring kidney biochemical parameters such as urinary excretion of total protein, blood urea nitrogen (BUN) and serum creatinine as well as histological analysis of the renal tissues. Moreover, ROS scavenging properties of quercetin were investigated by measuring oxidative stress biomarkers such as glutathione (GSH), thiobarbituric acid reactive substances (TBARS) levels, superoxide dismutase (SOD) and catalase (CAT) activities in rats treated with GM alone or combination of GM plus quercetin.

MATERIALS AND METHODS

Drugs and Chemicals Gentamicin sulfate was obtained from Memphis Co., for Pharm. and Chem. Ind. (Cairo, Egypt). Quercetin was supplied from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals used were of

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good quality and analytical grade.

Animals  Forty adult female Wistar albino rats weighing 150—200 g were selected for this study. The animals were obtained from Animal House, Faculty of Medicine, Assiut University (Assiut, Egypt), which were fed standard diet and water ad-libitum. During the study, rats were maintained with 12 h light/dark cycle in stainless steel metabolic cages to collect urine. Experimental protocols were approved by scientific research practice committee at Al-Azhar University, Assiut, Egypt.

Experimental Protocol  The animals were divided into 4 groups each of 10 rats: Group 1: Rats in this group were injected with normal saline, intraperitoneally and served as a control. Group 2: Rats in this group were orally treated with 50 mg/kg/d of quercetin for seven consecutive days.21,12) Quercetin was dissolved in ethanol and then diluted with tap water to the required concentration for oral administration. Group 3: Rats in this group were injected intraperitoneally with 80 mg/kg/d of gentamicin sulfate for 7 d.22,23) Group 4: Rats in this group were simultaneously treated with the same previous doses of quercetin and gentamicin for 7 d.21,12) Quercetin was dissolved in ethanol and then diluted with tap water to the required concentration for oral administration. Group 3: Rats in this group were injected intraperitoneally with 80 mg/kg/d of gentamicin sulfate for 7 d.22,23) Group 4: Rats in this group were simultaneously treated with the same previous doses of quercetin and gentamicin for 7 d. The kidneys of each animal were dissected out then fixed in buffered formalin for 12 h and processed for histopathological examination. Four micrometer-thick paraffin sections were stained with hema-toxylin and eosin for light microscope examination using conventional protocol.31) Other paraffin sections were stained with periodic acid-Schiff (PAS)32) to detect other pathologi-cal alterations clearly. A minimum of 8 fields for each kidney section were examined and assigned for severity of changes by an observer blinded to the treatments of the animals.

Statistical Analysis  Results were expressed as the means±S.E.M. Statistical significant difference was determined by one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test for multiple comparison. Probability values (p) less than 0.05 were considered to be statistically significant.

RESULTS

Kidney Function Tests  GM caused an elevation in urinary excretion of total protein levels (mg/24 h) from 12±1.73 to 70±5.7 after 7 d of its injection. In the animal group simultaneously treated with quercetin, the elevated urinary content of total protein was significantly reduced to 25±2.9 (mg/24 h) (Fig. 1a). Moreover, GM produced an elevation in blood urea nitrogen levels (BUN, mg/dl) from 26.6±2.3 to 53±2.8 (Fig. 1b) and serum creatinine levels (mg/dl) from 0.24±0.023 to 2.07±0.28 (Fig. 1c) after 7 d of its injection when compared with untreated control rats. On the other hand, simultaneous administration of quercetin plus GM significantly reduced the elevated BUN and serum creatinine levels to 32±2.29 mg/dl and 0.62±0.21 mg/dl respectively (Figs. 1b, c). Administration of quercetin alone had no effect on urinary excretion of total protein, BUN or serum creatinine (Figs. 1a—c).

Effects of Quercetin on Renal Oxidative Stress Biomarkers: GSH, TBARS Levels, CAT and SOD Activities  Glutathione (GSH) has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes; GSH is also a scavenger of hydroxyl radicals and singlet oxygen.33) In this study, GM produced a decrease in GSH levels (nmol/mg protein) from 20.9±1.15 to 9.4±1.7. Quercetin prevented the GM-induced decline in GSH content and restored its normal level to 18.24±1.16 (Fig. 2a). Free oxygen radicals can induce lipid peroxidation in cells, MDA is formed during oxidative degeneration and accepted as an indicator of lipid per-
oxidation. In this study, GM caused an elevation in lipid peroxide (TBARS) levels (nmol/mg protein) in renal tissues from 0.024±0.0023 to 0.073±0.004. Quercetin was able to normalize the elevated TBARS levels to 0.03±0.0028 (Fig. 2b). SOD (U/mg protein) and CAT activities (k/mg protein) were decreased in renal tissues of GM-treated rats from 18±1.73 to 8.66±1.45 (for SOD) and from 0.45±0.034 to 0.23±0.017 (for CAT). However, the reduced SOD and CAT activities were increased to 16±1.15 and 0.36±0.023 respectively after quercetin administration (Figs. 2c, d). Administration of quercetin did not show any significant effects on GSH, TBARS levels or SOD and CAT activities (Figs. 2a—d).

Histopathological Analysis Sections from control group showed normal histological structure of the glomeruli and renal tubules in the cortex (Fig. 3a) and normal tubules in the medulla (Fig. 3b). In renal sections from GM-treated rats, the glomeruli showed atrophy in some of them and hypertrophy in others (Fig. 3c). There were degeneration and necrosis in the epithelial cells lining the renal tubules with cystic luminal dilatation in others at the cortex (Fig. 3e). The endothelial cells lining the glomerular tufts showed swelling and there was intacytoplasmic vacuolation as detected by PAS (Fig. 3f).

Mononuclear leucocytes inflammatory cells infiltration was observed in focal manner between the tubules in the corticomedullary junction as well as in the perivascular area of the dilated blood vessels associated with edema (Fig. 3d). Quercetin reversed most of the histopathological alterations induced by gentamicin as seen from sections from GM-quercetin treated rats (Table 1). Photomicrographs from GM-quercetin group revealed normal glomeruli with absence of glomerular atrophy and hypertrophy (Fig. 3g) and (Table 1). Quercetin also alleviated the swelling of endothelial cells lining the glomerular tufts usually seen with GM (Fig. 3g) and (Table 1). In addition, quercetin reduced the mononuclear leucocytes inflammatory cells infiltration and alleviated the congestion in the adjacent blood vessels in the corticomedullary junction (Fig. 3h) and (Table 1). The rest of histopathological changes produced by GM were completely prevented by quercetin treatment (Table 1). In addition to its protective effects, quercetin alone was found to be safe and did not induce any histopathological changes in the kidney (Table 1). The histological alterations produced by GM that are ameliorated by quercetin are summarized in Table 1.

DISCUSSION

The aminoglycosides antibiotics including gentamicin are continuously being used in clinical practice because of their bactericidal efficacy against gram-negative bacterial infec-
tions, synergism with β-lactam agents, low cost and limited bacterial resistance. However, the incidence of nephrotoxicity from aminoglycosides has increased from 3% in 1969 to 20% in the past decade. In recent report, about 30% of patients treated with GM for more than 7 d show some signs of nephrotoxicity. The serious complications resulting from GM-induced nephropathy are limiting factors for its clinical usage. It has been shown that GM exerts its adverse renal effect by generation of ROS which results in severe tissue damage. Under normal conditions, ROS, which are generated during cellular functions, are eliminated by intrinsic antioxidant enzyme systems like superoxide dismutase, catalase and glutathione peroxidase. Therefore, reactive oxygen species scavengers and antioxidant molecules have the capacity to partially reduce or eliminate the deleterious effects induced by GM.

Quercetin, an important member of the flavonoid family, is a strong antioxidant agent found in vegetables and fruits, mainly in grape and red wine. It has been found that people living in southern parts of France are identified to have low incidence of coronary heart diseases and this was related to frequent consumption of Mediterranean diet containing quercetin and similar antioxidant flavonoids. Suzuki et al. have reported that oral quercetin has both gastric cytoprotective and gastric ulcer healing actions, at least in part, by scavenging free radicals generated in the injured or ulcerated area. Moreover, it has been found that quercetin has the capacity to protect the myocardial tissue against global ischemia and reperfusion injury due to its antioxidant and cytoprotective actions. In addition, Duarte et al. have re-

Fig. 3. Light Microscope Photomicrographs Showing the Protective Effects of Quercetin against Histopathological Alterations Induced by Gentamicin (GM) in the Kidney Tissues of Different Experimental Groups

Sections from control group showed normal histological structure of the glomeruli (g) in the cortex [photo (a)] and normal tubules (R) in the medulla [photo (b)], (H&E ×64). Renal sections from GM-treated rats, the glomeruli showed atrophy (arrow) in some of them and hypertrophy in others (G) [photo (c)], (H&E ×100). Leucocytes cells infiltration (m) was observed in focal manner between the tubules in the corticomedullary junction as well as in the perivascular area of the dilated blood vessels (V) associated with edema (O) [photo (d)], (H&E ×160). Degeneration and necrobiosis (D) in the epithelial cells lining the renal tubules with cystic luminal dilatation (C) in others at the cortex [photo (e)] (H&E ×64). The endothelial cells lining the glomerular tufts showed swelling and intercytoplasmic vacuolation (vc) as detected by PAS [photo (f)] (PAS ×140). Sections from GM-quercetin treated animals revealed normal glomeruli (arrow) [photo (g)], (H&E ×40). Few mononuclear leucocytes inflammatory cells infiltration (arrow) and congestion in the adjacent blood vessels (v) were observed in the corticomedullary junction [photo (h)] (H&E ×64).
In the present study, the role of ROS in GM-induced nephrotoxicity was assessed by the usage of antioxidant agent, quercetin, and further evaluation of alterations in the biochemical indicators of oxidative stress mainly GSH, enzymes and reduced the elevated levels of TBARS indicating of kidney function such as BUN, serum creatinine and total protein excretion in urine. Consistent with the data from the study of Pedraza-Chaverrí et al., we observed in our study that urinary excretion of total protein was increased after GM injection indicating tubular damage. On the other hand, BUN and serum creatinine levels were augmented indicating glomerular damage. However, the combined administration of quercetin plus GM to rats resulted in significant reduction in the elevated levels of urinary total protein concentrations, BUN and serum creatinine. These results could be in accord with several other researches, which reported that, compounds with antioxidant properties like S-allylmercaptopropionate, diallyl sulfide inhibited the increased urinary excretion of total protein induced by GM in rats. Other compounds like resveratrol, carnosine or garlic extract, partially prevented the increase in BUN and serum creatinine levels induced by GM.

In the present study, the role of ROS in GM-induced nephrotoxicity was assessed by the usage of antioxidant agent, quercetin, and further evaluation of alterations in the biochemical indicators of oxidative stress mainly GSH, TBARS levels, SOD and CAT activities beside histological changes. It has been reported that, quercetin exerts its antioxidant effects by scavenging free superoxide and hydroxyl radicals on one hand and by inhibiting xanthine oxidase activity and lipid peroxidation on the other.

Glutathione (GSH) has a very important role in protecting against oxygen free radicals by providing reducing equivalents for several enzymes; GSH is also a scavenger of hydroxyl radicals and singlet oxygen. In the present study, the levels of GSH in rat kidney tissues were significantly reduced after GM injection compared with control group. This result is confirmed by other studies, which have pointed to reduction of GSH levels after GM administration. An explanation to GSH depletion after GM treatment is increased consumption of GSH in non-enzymatic removal of oxygen-radicals. In addition, oxidation of GSH to GSSG by the oxidant stress, with efflux of GSSG being the major factor responsible for maintenance of the redox ratio. GSSG is of great biological importance, since it allows fine-tuning for the cellular redox environment under normal conditions and upon the onset of stress, and provides the basis for GSH stress signaling.

Sinha et al. have reported that galactosamine, an established experimental toxin, decreases the reduced glutathione (GSH) and enhances the renal tissue content of the oxidized form (GSSG). Pretreatment with antioxidant significantly increases the GSH and normalizes the GSSG levels in renal tissues. The conversion of GSSG to GSH is mediated through the enzyme glutathione reductase. Therefore, GM may act like S-(1,2-dichlorovinyl)-L-cysteine a known nephrotoxicant and interferes with the recycling of GSSG into GSH by inhibition of the enzyme glutathione reductase. Quercetin was reported to potentiate the activity of glutathione reductase under stress condition, an effect that may lead to enhancement of recycling of GSSG back to GSH. As a result, under oxidative stress reaction, the levels of GSH are higher in quercetin treated group than in other groups without quercetin treatment. Our results were consistent with this finding as simultaneous administration of quercetin plus GM significantly increased the GSH levels compared to that of GM-treated group only.

Moreover, GM causes rapid changes in membrane lipid composition. These changes of membrane lipid composition may be induced by free radical-initiated lipid peroxidation. This view is supported by increased MDA levels, one of the products of lipid peroxidation, in GM treated rats kidney.

We have found elevated lipid peroxide levels (TBARS) in the GM treated group, consistent with previous studies mentioned. On the other hand, the activities of SOD and CAT enzymes were greatly reduced in GM-treated rats compared with control group. The scavenging of superoxide radicals is achieved through an upstream enzyme, SOD, which catalyses the dismutation of superoxide to H₂O₂. This reaction has a 10000-fold faster rate than spontaneous dismutation. This reduction in SOD and CAT activities after GM injection has been previously recorded respectively, suggesting that oxidative stress is one of the causes of GM-induced renal damage. Interestingly, the combined administration of quercetin plus GM to rats reversed all of these alterations. Quercetin markedly, enhanced the activities of SOD and CAT enzymes and reduced the elevated levels of TBARS indicating that quercetin treatment decreases oxidative stress through its antioxidant properties.

Previous studies have shown that agents including gentamicin that enhance the generation of hydrogen peroxide and superoxide anion by mitochondria also enhances the generation of hydroxyl radical. Walker and Shah have examined the biological processes that may be affected by the hydroxyl radical generated from GM-treatment leading to acute renal failure. One of the mechanisms by which hydroxyl radical has been postulated to induce renal tissues damage by causing peroxidation of membrane lipids. This gives rise to membrane lipid damage and initiation of autocatalytic reactions. The damage in plasma membrane results in loss of os-
motric balance and intracellular calcium levels increase. Cellular swelling is the first manifestation of these reversible changes which can be detected under light microscope.\(^2\)\(^3\) Swelling of endothelium lining the glomerular tufts and tubular vacuolization is the reflection of these reversible changes in kidneys.\(^4\)\(^5\) In gentamicin group, we have observed swelling of endothelium lining the glomerular tufts and vacuolization in all samples. In GM-quercetin group, most of the glomeruli are normal in size and shape. Quercetin blocked cellular inflammatory process as indicated from reduction in mononuclear leucocytes inflammatory cells infiltration and alleviation of swelling of endothelium lining the glomerular tufts. Biochemical results were concordant with pathological findings since quercetin was able to normalize the elevated lipid peroxide (TBARS) levels and completely block lipid peroxidation. If intracellular free oxygen radicals increase, irreversible cellular injury process begins.\(^2\)\(^2\) Lysosomal enzymes activated and irreversible cell injury microscopically observed as tubular necrosis and tubular degeneration of kidney occurs.\(^4\)\(^3\) Scavenging of free oxygen radicals prevents irreversible renal cell injury and necrosis.\(^2\)\(^2\) We have observed tubular necrosis as a sign of irreversible injury in most sections examined from gentamicin group. Quercetin as an antioxidant inhibits lipid peroxidation and prevents renal cell injury manifested as tubular necrosis an irreversible cell damage. We have observed that quercetin treatment affected biochemical values and pathological findings, in accordance. Quercetin prevented the decrease in GSH levels, SOD and CAT activities and the increase in TBARS levels. In the same way, quercetin prevented reversible cell damage such as swelling of endothelium and vacuolization, and irreversible cell damage such as tubular necrosis. According to the pathological results, it can be stated that quercetin can be protective for degenerative injury caused by gentamicin treatment. Another mechanism underlying the antioxidative properties of flavonoids including quercetin is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membrane.\(^5\)\(^5\) These changes could strictly hinder the diffusion of free radicals and restrict their peroxidative reactions.

In conclusion, the present study revealed the nephrotoxic effects of GM. The use of quercetin in combination with GM minimized its toxicity as revealed from decreasing urinary excretion of total protein, BUN and serum creatinine levels. Oxidative stress reactions and ROS may be one of the mechanisms of GM-induced nephrotoxicity as indicated from alterations in oxidative stress biomarkers. The correction of oxidative stress biomarkers by quercetin was consistent with amelioration of the histopathological changes induced by GM. The ameliorative effect of quercetin against GM-induced renal damage may be at least in part due to its antioxidative and free radicals scavenger properties of quercetin.

REFERENCES