Protective effect of taurine and quercetin against renal dysfunction associated with the combined use of gentamycin and diclofenac

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The potential protective effects of taurine and quercetin against gentamycin (GM)/diclofenac (DC) combined nephrotoxicity were investigated in rats. The results showed that administration of DC alone at an oral dose of 5 mg/kg b.wt/day for 28 days had no significant effect on the measured parameters, except for marked increase in urinary uronic acid excretion. Administration of GM alone at a dose of 100 mg/kg b.wt/day i.p. for 8 days resulted in obvious nephrotoxicity. Combined GM-DC treatment led to the most pronounced nephrotoxicity, as indicated by greater elevations in serum urea, creatinine and urinary N-acetyl-β-D-glucosaminidase (NAG), together with severe depression of renal cortical Na⁺, K⁺-ATPase, compared to GM-treated group. Moreover, only combined treatment resulted in significant decrease in urinary potassium and renal cortical glutathione peroxidase (GSHPx), together with an increase in renal cortical lipid peroxidation products (LPOs). Co-administration of taurine or quercetin normalized creatinine clearance and ameliorated the elevations in urinary proteins, uronic acids, NAG and renal cortical LPOs in GM/DC treated rats. The study justifies the use of taurine and quercetin as renoprotective agents against the nephrotoxicity caused by GM/DC therapy.

**Keywords:** Nephrotoxicity, Gentamycin, NSAIDs, Taurine, Quercetin.

Evidences implicate the simultaneous use of multiple nephrotoxic drugs as a major risk factor for the development of severe renal impairment. Gentamycin (GM), the most commonly used aminoglycoside antibiotic is often prescribed to patients with infections and with other symptoms such as pain and inflammation; situations that require the application of non-steroidal anti-inflammatory drugs (NSAIDs). The frequent use of NSAIDs in combination with GM poses an additional risk of nephrotoxic renal failure. Nephrotoxicity is reported in as many as 10-26% of patients receiving aminoglycosides and in 16% of those receiving NSAIDs.

Although newer antibacterial agents have been developed, aminoglycosides remain among the most widely used antibiotics against life-threatening gram-negative infections, which may be attributed to their lower cost, greater effectiveness and longer experience with their use. Aminoglycosides are almost exclusively filtered by the glomerulus and excreted unchanged. However, a small but sizable proportion of the filtered drug (2-5%) undergoes proximal tubular reabsorption and sequestration in the lysosomes. Moreover, their high concentrations may be released into the cytosol and then interact with intracellular targets, causing disruption of a number of critical processes including mitochondrial respiration and microsomal protein synthesis.

NSAIDs have been found to cause various forms of renal deterioration. Their renal adverse effects are often associated with prolonged use, especially in high risk patients such as those suffering from heart, liver and pre-existing renal disease. NSAIDs produce most of their therapeutic effects through inhibition of prostaglandin (PG) synthesis by acting as reversible competitive inhibitors of cyclooxygenases (COX-1 and 2). Inhibition of COX-2 is thought to mediate, at least in part the antipyretic, analgesic and anti-inflammatory actions of NSAIDs. On the other hand, the simultaneous inhibition of COX-1 results in deleterious side effects such as gastrointestinal ulceration and nephropathy.

Taurine (2-aminoethanesulfonic acid) is reported to attenuate a variety of renal diseases occurring in different animal models as a result of excessive reactive oxygen species (ROS) formation such as...
puromycin aminonucleoside nephropathy and adriamycin-induced nephrotic syndrome. Moreover, bioflavonoids have attracted attention as a new class of renoprotective agents via their antioxidant activity. Among the tested bioflavonoids, quercetin is shown to have the highest antioxidant activity.

In the present study, we have investigated the effect of the NSAID diclofenac sodium in augmenting the nephrotoxic action of GM, in addition to evaluating the possible protective effect of taurine and quercetin on GM/diclofenac combined nephrotoxicity in rats. The various biochemical parameters such as serum and urinary electrolyte levels (sodium, potassium) serum urea, creatinine, and creatinine clearance levels, urinary protein, uronic acid, \( \gamma \)-glutamyl transferase (\( \gamma \)-GT) and N-acetyl-\( \beta \)-D-glucosaminidase (NAG) content/activity, in addition to kidney weight, renal cortical total phospholipids, lipid peroxidation products (LPOs), glutathione (GSH), Na,K-adenosine triphosphatase (Na,K-ATPase) and glutathione peroxidase (GSHPx) content/activity have been determined.

Materials and Methods

Animals
Male albino rats (n=80) of Wistar strain weighing 180-220 g were obtained from the farm of the National Institute for Vaccination, Helwan. The animals were housed in plastic cages maintained under controlled environmental conditions and fed a standard chow diet and water ad libitum throughout the experimental work.

Chemicals
All drugs, biochemical reagents, standard enzymes, substrates and coenzymes, unless otherwise specified were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Other chemicals and organic solvents were of Analar grade.

Experimental design
Rats were randomly divided into five experimental groups, each of 10 rats, in addition to control groups. The group I was injected i.p. with GM [rifobacin injections (40 mg/ml); Galaxowellcome, Egypt] at a dose of 100 mg/kg b.wt/day for 8 days. The group II received diclofenac sodium (Novartis Pharma, Egypt) orally at a dose of 5 mg/kg b.wt/day for 28 days. The group III started treatment with diclofenac (DC) as in the group II for 28 days and received an additional treatment with GM as in the first group during the last 8 days of DC treatment. The groups IV and V were treated in a similar manner to the group III, but received a further i.p. injection with either taurine (dissolved as a 10% aqueous solution) at a dose of 0.75 g/kg b.wt/day or quercetin (dissolved as a 200 mg% solution in dimethylsulfoxide (DMSO)) at a dose of 2 mg/kg b.wt/day concurrently throughout the treatment period. A normal control group (n = 6) was run with each treated group and received the vehicle(s), in which the drugs and/or modulators were dissolved by the same route. All injections were carried out between 9.00-10.00 a.m. to minimize the circadian variation in GM nephrotoxicity.

Collection of urine
After the last injection, animals from each group were kept individually in wire-bottom stainless steel metabolic cages for the collection of 24 h urine samples. During the period of urine collection, animals were fasted and allowed free access to water only. The volumes of the collected urine samples were measured and recorded. Urine specimens were centrifuged at 600 \( \times \) g for 15 min to sediment debris and particles. The supernatants were divided into aliquots and stored at -20°C until analyzed for creatinine, electrolytes, proteins, uronic acids, \( \gamma \)-GT and NAG.

Blood and tissue sampling
At the end of each treatment period, the animals were sacrificed by decapitation. The blood of each animal was collected in a dry centrifuge tube and allowed to clot, then centrifuged at 600 \( \times \) g for 15 min for serum separation. Aliquots of the separated serum were stored at -20°C for subsequent determination of urea, creatinine and electrolytes.

Immediately after decapitation, rats were dissected for isolation of the two kidneys. Renal capsules were stripped-off and the kidneys were washed by ice-cold isotonic saline, blotted between filter papers and weighed. Each kidney was longitudinally divided into two bean-shaped halves. The renal cortices of both kidneys were rapidly dissected free on ice, weighed and then homogenized in ice-cold redistilled water using an ice-jacketed teflon homogenizer (Potter-Elvehjem type) to obtain a 10% aqueous homogenate. An aliquot of the homogenate was kept at -20°C for subsequent determination of total phospholipids.

Another aliquot was mixed with an equal volume of ice-cold 2.3% KCl solution, then centrifuged at
1000 × g for 15 min at 4°C (Dupont Sorvall ultracentrifuge, USA) and the obtained post-nuclear supernatant was stored at -20°C and used for determination of LPOs. A third aliquot of the homogenate was mixed with an equal volume of ice-cold 4% sulfosalicylic acid, centrifuged at 30,000 × g for 15 min at 4°C and the supernatant was assayed for cold 4% sulfosalicylic acid, centrifuged at 30,000 × g for 15 min at 4°C and the supernatant was assayed for GSH. Finally, a fourth aliquot of the homogenate was mixed with an equal volume of ice-cold sucrose-imidazole buffer, pH 7.2 containing 50 mM imidazole, 2 mM EDTA and 0.6 M sucrose and centrifuged at 15,000 × g for 15 min at 4°C. The obtained post-mitochondrial supernatant was further centrifuged at 105,000 × g for 45 min at 4°C. The separated supernatant (cytosolic fraction) was stored at -20°C and used for estimation of GSHPx activity while the remaining pellet (microsomal fraction) was resuspended in one third of the original volume of sucrose-imidazole buffer (pH 7.2) by vigorous agitation with a vortex mixer to ensure complete resuspension, then stored at -20°C and used for estimation of Na, K-ATPase activity.

Biochemical studies

Serum urea was determined by an enzymatic method based on the urease-modified Berthelot reaction using a kit provided by bioMérieux (France). Creatinine was estimated in serum and urine (diluted 1:100) by the alkaline picrate method. Creatinine clearance was calculated by the standard equation and expressed as ml/min/100 g body wt. Serum and urinary sodium was measured by the colorimetric magnesium-uranyl acetate method using a kit provided by QCA (Spain). Determination of potassium in serum and urine was done using the turbidimetric tetraphenylborate (TPB) method using a kit provided by QCA (Spain).

Total protein in urine was estimated as described previously. Urinary uronic acid concentration was determined by the method described elsewhere which is based on the appearance of a chromogen when uronic acids are heated to 100°C with conc. H₂SO₄/tetraborate reagent, and then treated with m-hydroxydiphenyl. A urine blank was run with each urine sample to correct for the pinkish colour produced by carbohydrates with conc. H₂SO₄/tetraborate at 100°C.

Urinary γ-GT activity was determined by a kinetic colorimetric method using a kit provided by bioMérieux (France). This method depends on measuring the rate of formation of the yellow p-nitroaniline measured at 405 nm which is proportional to the γ-GT activity in the sample. Enzyme activity was expressed as unit/mmol creatinine to compensate for the variation in urine concentration. One unit of γ-GT activity was defined as the amount of enzyme that catalyzed the formation of 1 µmole of product (p-nitroaniline) per min under the assay conditions. Urinary NAG activity was expressed as unit/mmol creatinine to compensate for the variation in urine concentration. One unit of NAG activity was defined as the amount of enzyme required to produce 1 µmole of PNP per min under the assay condition.

The determination of renal cortical total phospholipids involved two steps: the extraction of total lipids and the estimation of total phospholipids by a simple colorimetric method which is based on heating the chloroformic phospholipid extract with a chromogenic solution that is a modification of a spray reagent formulated by Vaskovsky and Kostetsky. The chromogenic solution reacts directly with the phospholipid phosphorus to form a Prussian blue complex. Renal cortical LPOs were measured by the thiobarbituric acid assay. The concentration of LPOs was expressed as nmoles equivalents of malondialdehyde (MDA)/g wet tissue.

The determination of renal cortical Na,K-ATPase activity involved pre-incubation of the microsomal fraction with sodium deoxycholate (DOC) under the conditions found optimal for demasking all the latent Na,K-ATPase activity in the preparation. The activity of Na,K-ATPase was assayed in the DOC-activated microsomal fraction. Total ATPase activity was estimated as the inorganic phosphorus (Pi) released after incubation of the sample with ATP in presence of Na⁺, K⁺ and Mg²⁺ ions and in absence of ouabain. Mg-ATPase activity was estimated as the amount of Pi released in presence of ouabain. Na,K-ATPase activity was calculated by subtraction. The liberated Pi was estimated colorimetrically. The enzymatic activity was expressed as µmoles Pi released/h/mg protein. Renal cortical GSH was determined in the protein-free homogenate using the method of Ellman as modified by Engelhardt and...
Homma\textsuperscript{15} and expressed as μmole/g wet tissue. The activity of renal cortical GSHPx was determined by the direct spectrophotometric method\textsuperscript{36} and expressed as μmoles NADPH oxidized/min/mg protein. The protein content in the cytosolic and microsomal fractions was determined by the method of Lowry et al.\textsuperscript{37}.

Statistical analysis

Data from control and treated groups were evaluated by One-way analysis of variance (ANOVA). If the F values were significant, Tukey-Kramer multiple comparison test was used to compare the treated and the control groups. The level of significance was accepted at $P < 0.05$.

Results

Kidney function tests

The data shown in Table 1 showed that DC administration had no significant effect on the measured kidney function tests. On the other hand, administration of GM either separately or in combination with DC resulted in significant increase in serum urea and creatinine levels ($p<0.001$), accompanied by markedly reduced creatinine clearance. The increase in serum urea and creatinine levels caused by GM-DC combination was significantly greater than that caused by GM alone ($p<0.001$). Co-administration of either taurine or quercetin with GM-DC markedly reduced the elevated serum urea and creatinine levels and almost normalized the lowered creatinine clearance ($p<0.001$).

Serum and urinary electrolyte levels

Table 1 revealed that DC administration did not change the measured electrolyte levels. Serum sodium levels were significantly increased in GM and GM-DC groups ($p<0.001$). Neither serum potassium nor urinary sodium was affected. On the other hand, urinary potassium was significantly decreased ($p<0.05$) only by the combined treatment. Taurine co-administration failed to correct the elevated serum sodium or the lowered urinary potassium level observed in GM-DC-treated group. On the other hand, quercetin attenuated the hypernatremia produced in that group ($p<0.05$). However, it could not modify the lowered urinary potassium level.

Urinary protein and uronic acid levels

As illustrated in Table 1, DC administration had no effect on urinary protein level. However, it caused a marked elevation in urinary uronic acid level ($p<0.001$). GM administration either separately or in combination with DC resulted in marked elevation in urinary protein and uronic acid levels ($p<0.001$). Co-administration of either taurine or quercetin with GM-DC attenuated the observed elevations in urinary protein and uronic acid concentrations ($p<0.001$).

Urinary enzyme activities

The data given in Table 1 showed that DC administration had no significant effect on the measured urinary enzyme activities. On the other hand, administration of GM either alone or in combination with DC resulted in a two-fold increase in urinary γ-GT activity ($p<0.001$) and a substantial NAG enzymuria reaching to 10- and 22-times the normal control level, respectively ($p<0.001$). Co-administration of either taurine or quercetin with GM-DC significantly reduced the elevated levels of the two enzymes ($p<0.001$).

Kidney weight and renal cortical total phospholipid content

From Table 1, it was evident that DC administration did not affect kidney weight or renal cortical total phospholipids content. However, they were significantly increased in GM and GM-DC groups ($p<0.001$). Co-administration of either taurine or quercetin with GM-DC could not significantly reduce the increased kidney weight. However, taurine almost normalized the elevated renal cortical total phospholipids, while quercetin reduced such elevated level ($p<0.01$). Indeed, a positive correlation was found between urinary NAG activity and renal cortical total phospholipid content in GM-DC and quercetin-co-treated groups (Fig. 1).

Renal cortical LPOs content and Na,K-ATPase activity

The data shown in Table 1 demonstrated that administration of DC or GM separately had no effect on renal cortical LPOs. GM only caused 52% inhibition in Na,K-ATPase activity as compared to the normal control ($p<0.001$). However, combined administration of GM-DC resulted in a significant elevation ($p<0.001$) in renal cortical LPOs and a marked inhibition ($p<0.001$) of renal cortical Na,K-ATPase activity. Co-administration of either taurine or quercetin with GM-DC led to 31% and 27% decrease in LPOs respectively ($p<0.001$). Furthermore, both modulators significantly elevated the depressed Na,K-ATPase activity ($p<0.001$). A negative correlation was found between LPOs and
Table 1—Protective effects of taurine (TA) or quercetin (QU) against the biochemical changes induced by gentamycin (GM) when given in combination with diclofenac (DC).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal control</th>
<th>GM</th>
<th>DC</th>
<th>GM + DC</th>
<th>TA+GM+DC</th>
<th>QU+GM+DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea (mg/dl)</td>
<td>40.49 ± 3.73</td>
<td>79.26 ± 5.36*</td>
<td>54.62 ± 7.21 (8)</td>
<td>108.80 ± 20.54*</td>
<td>62.39 ± 14.37*</td>
<td>59.11 ± 8.53*</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.13 ± 0.11</td>
<td>1.50 ± 0.16*</td>
<td>1.13 ± 0.14 (10)</td>
<td>2.20 ± 0.62*</td>
<td>1.21 ± 0.20*</td>
<td>1.50 ± 0.19*</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/100g b.wt)</td>
<td>0.25 ± 0.02 (11)</td>
<td>0.16 ± 0.04* (9)</td>
<td>0.23 ± 0.02 (10)</td>
<td>0.12 ± 0.04*</td>
<td>0.23 ± 0.03*</td>
<td>0.22 ± 0.03*</td>
</tr>
<tr>
<td>Serum sodium (mmole/l)</td>
<td>147.5 ± 22.1 (10)</td>
<td>193.6 ± 27.5* (9)</td>
<td>161.2 ± 18.4 (9)</td>
<td>198.9 ± 18.1*</td>
<td>177.9 ± 13.6*</td>
<td>167.7 ± 13.3*</td>
</tr>
<tr>
<td>Serum potassium (mmole/l)</td>
<td>6.38 ± 0.53 (12)</td>
<td>6.57 ± 0.83 (10)</td>
<td>6.80 ± 0.84 (9)</td>
<td>6.96 ± 0.66</td>
<td>6.28 ± 0.49</td>
<td>6.15 ± 0.48</td>
</tr>
<tr>
<td>Urinary sodium (mmole/day)</td>
<td>1.09 ± 0.13 (10)</td>
<td>0.96 ± 0.14 (10)</td>
<td>1.01 ± 0.17 (10)</td>
<td>0.88 ± 0.09</td>
<td>1.04 ± 0.15</td>
<td>1.00 ± 0.18</td>
</tr>
<tr>
<td>Urinary potassium (mmole/day)</td>
<td>1.43 ± 0.23 (8)</td>
<td>1.35 ± 0.30 (9)</td>
<td>1.13 ± 0.24 (10)</td>
<td>1.07 ± 0.29*</td>
<td>1.16 ± 0.22</td>
<td>1.23 ± 0.22</td>
</tr>
<tr>
<td>Urinary proteins (mg/day)</td>
<td>6.00 ± 1.10 (12)</td>
<td>26.51 ± 6.40* (9)</td>
<td>7.91 ± 2.15 (10)</td>
<td>25.43 ± 7.04*</td>
<td>16.12 ± 3.49*</td>
<td>14.01 ± 3.93*</td>
</tr>
<tr>
<td>Urinary uronic Acids (mg/day)</td>
<td>1.89 ± 0.23 (12)</td>
<td>4.94 ± 1.09* (9)</td>
<td>3.93 ± 0.87* (9)</td>
<td>5.60 ± 0.98*</td>
<td>2.51 ± 0.74*</td>
<td>2.65 ± 0.60*</td>
</tr>
<tr>
<td>Urinary γ-GT (U/m mole creatinine)</td>
<td>72.55 ± 12.48 (10)</td>
<td>161.51 ± 34.12* (7)</td>
<td>90.92 ± 17.92 (8)</td>
<td>170.39 ± 35.60*</td>
<td>100.32 ± 25.02*</td>
<td>82.61 ± 13.95*</td>
</tr>
<tr>
<td>Urinary NAG (U/m mole creatinine)</td>
<td>1.06 ± 0.15 (8)</td>
<td>10.20 ± 2.69* (9)</td>
<td>0.94 ± 0.26 (10)</td>
<td>23.42 ± 6.42*</td>
<td>10.18 ± 2.69*</td>
<td>7.19 ± 1.61*</td>
</tr>
<tr>
<td>Kidney weight (g/100 g b. wt)</td>
<td>0.60 ± 0.04 (12)</td>
<td>0.74 ± 0.09* (10)</td>
<td>0.67 ± 0.12 (10)</td>
<td>0.77 ± 0.07*</td>
<td>0.72 ± 0.06*</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td>Total phospholipids (mg/g tissue)</td>
<td>20.31 ± 3.78 (12)</td>
<td>32.08 ± 3.36* (10)</td>
<td>17.99 ± 4.91 (10)</td>
<td>35.81 ± 6.99*</td>
<td>23.82 ± 3.97*</td>
<td>27.00 ± 6.29*</td>
</tr>
<tr>
<td>LPOs (n mole equivalents MDA/g tissue)</td>
<td>47.30 ± 4.60 (10)</td>
<td>55.53 ± 10.28 (9)</td>
<td>54.60 ± 8.49 (10)</td>
<td>77.81 ± 11.95*</td>
<td>53.93 ± 7.03*</td>
<td>56.92 ± 6.44*</td>
</tr>
<tr>
<td>Na,K-ATPase (µmole Pi/h/mg protein)</td>
<td>31.22 ± 5.83 (12)</td>
<td>14.88 ± 3.50* (10)</td>
<td>29.56 ± 6.11 (8)</td>
<td>6.39 ± 1.53* !</td>
<td>27.26 ± 5.23*</td>
<td>20.09 ± 4.76* !</td>
</tr>
<tr>
<td>GSH (µmole/g tissue)</td>
<td>3.14 ± 0.19 (12)</td>
<td>2.86 ± 0.19 (10)</td>
<td>3.14 ± 0.33 (10)</td>
<td>2.82 ± 0.32</td>
<td>3.14 ± 0.46</td>
<td>3.10 ± 0.37</td>
</tr>
<tr>
<td>GSHPx (µmole NADPH oxidized/min/mg protein)</td>
<td>0.59 ± 0.08 (11)</td>
<td>0.52 ± 0.05 (7)</td>
<td>0.56 ± 0.06 (10)</td>
<td>0.45 ± 0.04*</td>
<td>0.52 ± 0.07</td>
<td>0.52 ± 0.07</td>
</tr>
</tbody>
</table>

Significant difference from normal control at $p < 0.05^6$, 0.01, 0.001*
Significant difference from (GM+DC)-treated group at $p < 0.05^5$, 0.01, 0.001*
Significant difference from GM-treated group at $p < 0.05^2, 0.001^7$

Na, K-ATPase activity in the renal cortex of rats receiving the drug combination, taurine as well as quercetin (Fig. 2).

Renal cortical GSH content and GSHPx activity

The renal cortical GSH content was not modified by any of the used drugs or modulators (Table 1). Only the combined administration of GM and DC significantly inhibited the renal cortical GSHPx activity ($p<0.001$). Co-administration of either taurine or quercetin with GM-DC resulted in an apparent increase in GSHPx activity, as compared to GM-DC-treated group that was insignificantly different from the normal control level.
Discussion

The treatment of rats with DC alone had no significant effect on the kidney, except for a marked increase in urinary uronic acid excretion. These findings were in agreement with previous study that the toxic effects of NSAIDs were limited to situations of pre-existing renal damage, or when the drug is administered in combination with other nephrotoxic drugs\(^5\). The elevation in uronic acid excretion could be the product of an early sub-toxic renal degeneration process that involved the breakdown of renal interstitial ground substance with the loss of renal mucopolysaccharides\(^3\).

The elevated serum urea and creatinine levels, together with the lowered creatinine clearance in GM-treated rats were in agreement with previous findings\(^3\). These results could be attributed to reduction in glomerular capillary ultrafiltration coefficient (K\(_f\)) and/or tubular necrosis with a subsequent decrease in the number of functioning nephrons and decline in glomerular filtration rate (GFR)\(^1\). The later could also explain the elevated serum sodium level by GM as a decrease in functioning nephrons number could trigger multiple adaptive processes in the hyperfunctioning remaining nephrons, including augmented rates of sodium reabsorption\(^4\).

The observed proteinuria in GM-treated group could result from diminished tubular reabsorption of normally filtered small molecular weight protein, such as β\(_2\)-microglobulin. Alternatively, it may be a consequence of GM-induced proximal tubular necrosis and loss of brush border forming urinary protein casts\(^4\). The accelerated turnover of the brush border membrane as a result of deleterious interactions between GM and membrane phospholipids\(^4\) might also explain the observed increase in urinary γ-GT, a brush border membrane enzyme. On the other hand, NAG enzymuria, following GM treatment might be derived from leakage of the lysosomal enzymes, due to alterations in lysosomal membrane integrity\(^4\). As an extension to the interaction of GM with membrane phospholipids, renal cortical Na,K-ATPase was depressed which could also be due to interference with mitochondrial ATP production\(^4\).

Evidences suggest that GM-induced phospholipidosis is due to decreased catabolism of phospholipids which leads to their accumulation within lysosomes in the form of myeloid bodies\(^4\). Indeed, GM is reported to depress the activities of lysosomal phospholipases A and C, as well as sphingomyelinase in rat renal cortex\(^3\). The observed increase in the kidney weight of GM-treated rats could be related to such elevation in the renal cortical total phospholipid content. It may be also a consequence of renal cell swelling as a result of drug-induced inflammatory reaction\(^1\).

There are a number of conflicting reports about the role of ROS in GM nephrotoxicity\(^4\). Nevertheless, the absence of detectable oxidative stress in our model was compatible with the fact that GM is a redox-inactive substance not metabolized in the body and excreted in urine unchanged\(^4\).

Higher degree of nephrotoxicity was markedly noticed upon co-administration of GM with DC. This was evident from the greater elevation in serum urea and creatinine levels and urinary NAG excretion. The potentiation of GM nephrotoxicity by concomitant DC treatment may be best explained in view of the fact that PGs play a major role in regulating functional responses to the tubulotoxic drug GM. NSAIDs, due to their inhibitory action on PG synthesis worsen the renal toxicity of aminoglycoside.
antibiotics. In addition, the inhibition of PG synthesis is supposed to interfere with PG-mediated renin release with consequent suppression of the renin-aldosterone axis, resulting in the reduction of potassium excretion by the distal nephron.

Only in the combined treatment, oxidative stress was observed by increase in LPOs, which was correlated with depression in GSHPx activity. This could have resulted in the accumulation of hydrogen peroxide. In addition, the reduction in the number of functioning nephrons leads to increased metabolic activity by the remaining nephrons with excessive oxygen consumption. This could lead to enhanced generation of ROS and consequently LPO and depression in the renal cortical Na,K-ATPase activity in GM-DC-treated group. ROS could modify the active site of the membrane-bound enzyme Na,K-ATPase. In this regard, a negative correlation was found between LPOs and Na,K-ATPase activity in those rats.

Taurine exhibited marked renal protection as evident by lowering of serum urea, normalization of serum creatinine and creatinine clearance, reduction in urinary protein and uric acid excretion, amelioration of enzymuria, suppression of renal cortical phospholipid accumulation and lipid peroxidation, as well as improved renal cortical Na,K-ATPase activity as compared to the GM-DC treated group. These results confirmed previously reported findings.

Taurine has been demonstrated to enhance renal kallikrein synthesis and consequently, increases the availability of vasodilatory kinins. Bradykinin is shown to exert its effect on renal O2 consumption through dependence on nitric oxide synthase (NOS) activity. Nitric oxide (NO), a potent vasodilator is an important regulator of renal hemodynamics and that produced by endothelial isoenzyme (eNOS) is the prime regulator. In addition, taurine as an antioxidant is expected to increase the availability of NO, since oxidative stress depletes NO via scavenging by superoxides. This possibly leads to the increase in renal blood flow (RBF) and GFR, thus enhancing the urinary excretion of urea and creatinine. Also, the activation of renal kallikrein-kinin system by taurine is reported to attenuate GM-induced proximal tubular damage with the consequent reduction in brush border loss and protein cast formation. Taurine also prevents the formation of myeloid bodies. Again, this could be related to the activation of renal kallikrein-kinin system, since bradykinin is shown to enhance the activity of phospholipase A2 in endothelial cells, leading to increased metabolism of phospholipids.

Taurine by interaction with the neutral phospholipids of biological membranes can protect the membrane organization against free radical attack. Thus, renal cortical membrane-bound Na,K-ATPase activity was restored in taurine co-treated group. This was confirmed by the negative correlation between renal cortical LPOs and Na,K-ATPase activity in this group. Furthermore, the renoprotective effect of taurine against the degenerative effects of GM-DC combination could be achieved via other mechanisms, including calcium modulation, osmoregulation and preservation of membrane integrity. The latter effect may be responsible for reducing the excretion of the membrane-bound enzyme γ-GT and the lysosomal enzyme NAG in urine.

Quercetin co-treatment ameliorated the biomarkers of kidney function. Urinary protein and uric acid excretion, γ-GT and NAG enzymuria, renal cortical phospholipid and LPOs contents were reduced while Na,K-ATPase activity was improved in comparison with GM-DC-treated group. The ability of quercetin to decrease arginine consumption in urea synthesis by inhibiting hepatic arginase makes arginine more available for the synthesis of proteins, polyamines and NO. Such effect would enhance renal regenerating capabilities, abolish the augmented tubular sodium reabsorption by the hyperfunctioning remaining nephrons and decrease vascular resistance, thus improving RBF and GFR.

Quercetin exhibits anti-inflammatory action through the inhibition of leukotriene synthesis and histamine release. Consequently, it decreases urinary uric acid excretion caused by the mucopolysaccharide degradation during inflammation. The decreased release of γ-GT and the lysosomal NAG enzymes in urine upon quercetin administration is related to its reported membrane stabilizing effect. Moreover, quercetin interacts with the polar head groups of membrane phospholipids, reducing GM binding to its membrane phosphatidylinositol receptor. This effect prevents the accumulation of GM within the lysosomes and the secondary inhibition of lysosomal phospholipases and phospholipidosis. The antiperoxidative effect of quercetin as a result of scavenging free radicals and chelating iron was exerted without modifying the activity of GSHPx and...
was in agreement with the results of previous study. As a consequence of LPOs inhibition, renal cortical Na,K-ATPase activity was improved by quercetin co-treatment.

Conclusion
The findings of the present study strongly suggest that the frequent use of NSAIDs should be considered as a potential risk factor that increases the susceptibility of the kidney to the toxic action of aminoglycosides. Furthermore, the study justifies the use of taurine and quercetin as renoprotective agents against the nephrotoxicity caused by combined GM-DC therapy.

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