MORINDACITRIFOLIA EXTRACT ATTENUATES GENTAMICIN INDUCED RENAL DAMAGE AND OXIDATIVE STRESS IN RATS

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ABSTRACT
Reducing excess duration of antibiotic therapy is a strategy for limiting the spread of antibiotic resistance, but altering physician practice to accomplish this requires knowledge of the factors that influence physician antibiotic choice (Hassan et al., 2008). Clinically effective antimicrobial agents exhibit selective nephrotoxicity, with varying degrees, which is characterized by necrosis of the epithelial cells lining the tubules of kidney. There are a number of methods available for the investigation of the anatomical and functional status of the kidneys. These include radiography, biopsy and the calculation of ‘clearance values’ for various compounds. Gross kidney damage can often be detected by determining the degree of proteinuria, pH, glucose, albumin and by examining the urinary sediments. However, if only slight renal damage has occurred, the histological examination of the kidneys may be the only method available. The value of urinary enzymes as a non-invasive test for renal injury in toxicology and in medicine has been well documented. The diagnostic potential of urinary enzymes is often enhanced by the simultaneous assay of more than one enzyme, particularly if the activities of the enzymes used are high in different regions of the nephron. Many enzymes are released from the kidney tissue into the urine, when the kidney is injured. Many reports have appeared pertaining to changes in the activity of urinary enzymes induced by nephrotoxic drugs in animals, especially in rat.

KEY WORDS: Morindacitrifolia, Kidney, Gentamicin, Nephroprotective, Rat.
INTRODUCTION
The kidney is especially vulnerable to the hazards of potential drug toxicity because, firstly, it has a rich blood supply, which for a total mass of only about 0.4% of body weight, can amount to 25% of cardiac output. Secondly, the renal tubular cells may therefore become exposed to drug concentrations which far exceed those encountered in any other body tissue. Thirdly, the function of the kidney as an obligatory route for the elimination of certain drug means, if extra renal mechanisms of elimination are not activated, drug accumulation will occur. The higher concentrations of drugs in the circulation make the kidney more susceptible to direct damage, as in the case of nephrotoxicity of certain antimicrobials, especially amino glycosides (Bledsoe et al., 2008).

Aminoglycosides
The aminoglycosidic antibiotics have established their position as highly potent and valuable drugs against infections caused by a wide variety of Gram-negative and Gram-positive bacteria. (Hawkey and Finch, 2007). Aminoglycosides are structurally composed of aminosugars bound by glycosidic linkages (Kudo et al., 2005). The number and nature of the aminogroups differentiate each type of aminoglycoside (Carmi Bartal, 2003). The first and foremost identified and isolated aminoglycoside was Streptomycin from Streptomyces griseus and there are a large number of aminoglycosides available with variations in central ring and its substitutions.

a. Streptomycin – the central ring is streptone and the amino sugar is glucosamine.

b. The central ring is deoxystreptone in neomycin, kanamycin and gentamicin and the aminosugar being pentose and glucosamine for neomycin, kanosamine for kanamycin and garosamine for gentamicin.

c. The central ring is actinamide – spectinomycin.

Gentamicin
Structure and Uses
Gentamicin is a broad-spectrum aminoglycosidic antibiotic produced by the actinomycete, Micromonospora purpurea, which was first isolated in 1963. It consists of three major components and numerous minor ones. Gentamicin is one of the few heat-stable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of certain microbiological growth media. The major components of gentamicin C1, C2 and C1a which differ from each other by the degree of methylation at the 6’ position
Chemically gentamicin C₁A is 0-3-Deoxy-4-C-methyl-3-(methylamino)-β-Larabinopyranosyl-(1→6)-0-[2,6-diamino-2,3,4,6-tetradecoxy-a-D-erythrohexopyranosyl-(1→4)]-2-deoxy-D-streptamine. A minor component closely related to the garamicins C’s are C₂bA, A₁, A₂, a₃, B, X₂, JI-20 A, JI-20B have been well established. Mass spectral analysis of the structure of gentamicin indicates the presence of aminosugargarosamine and 2-deoxystreptamine.

**Adverse Effects of Gentamicin**

Gentamicin, although having established its position as highly potent and valuable drug in the treatment of infections due to a wide variety of gram-negative and gram-positive bacteria, a narrow range exists between its therapeutic to toxic serum concentrations. The clinical application of gentamicin is limited chiefly by the development of nephrotoxicity (Banday et al., 2008). The primary site of aminoglycoside – induced renal injury is at the proximal tubular cells. Nephrotoxicity due to exposure of gentamicin have been well documented in both experimental animals (Chiu et al., 2008) and in clinical trials (Soliman et al., 2007).

**Binding and Uptake of Gentamicin to Proximal Tubular Cells**

The interaction between the aminoglycoside - gentamicin and the brush border membrane of the renal proximal convoluted tubules have been described in rats (Hirode et al., 2008) and in rabbits (Hancock et al., 2005). Gentamicin is polybasic due to their side chains containing amino groups and thus is polycationic at physiological pH. Hence the acidic anionic phospholipids of the plasma membrane are prime targets for the charge interaction with the gentamicin (Baronas et al., 2007). Several lines of evidence indicate, particularly phosphoinositides like phosphoinositol (PT), phosphatidylinositol-4, 5-biphosphate (PI-P₂), to be an integral component of the gentamicin binding sites. Of the phosphoinositides, PI-P₂ has been shown to have the highest binding affinity for gentamicin followed by PIP and PI (Ogwan et al., 1993). The binding of the drug to the receptor is followed by pinocytosis of the drug-receptor complex with subsequent translocation of the complex to secondary lysosomes. Within the lysosomes, gentamicin might interfere with the catabolism of the receptor by directly inhibiting phospholipase A and C or by modifying substrate – enzyme affinity or by raising the intralysosomal pH above the effective range of the enzyme. Inhibition of phospholipase C, results in cortical phospholipidosis, characterized as electron dense phosphatidyl inositol rich-myeloid bodies within lysosomes (Konapska et al., 2007, Dai et al., 2006 and Kizawa et al., 2003). The measurement of other parameters such as urinary pH,
glucose, urea and the cells in urine, have found their place in the diagnostic importance of gentamicin induced toxicity (Sener et al., 2002).

MATERIALS AND METHODS

Experimental Induction of Nephrotoxicity

Gentamicin was chosen to induce nephrotoxicity in rats. Poon et al. (2007) have shown that gentamicin at a concentration of 100 mg/kg/bw is nephrotoxic to rats. Hence, this dosage was chosen for intraperitoneal administration for 10 days.

Experimental setup

Rats were divided into four groups comprising of six animals each.

Group I  -  Served as control
Group II  -  Received *Morindacitrifolia* extract (500 mg/kg/bw/po) by oral gavage for 10 days.
Group III -  Administered gentamicin (100 mg/kg/bw) intraperitoneally (ip) for 10 days.
Group IV  -  Received simultaneously both *Morindacitrifolia* and gentamicin.

Collection of Rat Urine

On the day before sacrifice, rats were housed in metabolic cages for 24 hr urine collection. The urine was free from faecal contamination. The rats were provided with water but no feed was given. A 50 ml beaker maintained at 0°C in ice-bath was used for urine collection.

(a) A portion of the sample was acidified with concentrated HCl and used for the analysis of creatinine and urea.
(b) The remaining portion was dialysed at 4°C against distilled water for 3hr. Aliquots of the dialysed urine were then used for the determination of enzymes and protein.
(c) 1 ml of 24 hr urine was taken and to this 0.5 ml each of HNO₃and perchloric acid were added. The contents were digested over a sandbath until it became colorless and was made upto a known volume with deionised water. Aliquots of this were used for estimation of electrolytes (Ballentine and Burford, 1957).

METHODS

Creatinine was estimated by the method of Owen et al., (1954). The protein content in urine was estimated by the method of Lowry et al. (1951). The total protein content is expressed as mg/24 hr urine. Urea was estimated by the method of Natelson (1957) using
diacetylmonoxime reagent. Phospholipids were estimated by the method of Rouser et al., (1970).

**ESTIMATION OF ELECTROLYTES**

Atomic absorption spectrophotometer (Model : GBC 906 AA) was used for the estimation of calcium and magnesium.

**ESTIMATION OF CALCIUM**

**Standard** : Calcium carbonate (1000 µg/ml)

2.497 g of dried calcium carbonate was dissolved in 25 ml of 1N HCl and diluted to 1 litre to give a solution containing 1000 µg of calcium/ml.

<table>
<thead>
<tr>
<th>Light source</th>
<th>Hollow cathode lamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamp current</td>
<td>10.0 mA</td>
</tr>
<tr>
<td>Flame type</td>
<td>Nitrous oxide-acetylene (oxidizing)</td>
</tr>
</tbody>
</table>

![Table: Calcium Estimation Parameters](image)

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Slit width (nm)</th>
<th>Working range (µg/ml)</th>
<th>Sensitivity (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>422.7</td>
<td>0.5</td>
<td>1-4</td>
<td>0.02</td>
</tr>
</tbody>
</table>

For nitrous oxide-acetylene, an ionization buffer (2000 µg/ml potassium) was used. Values are expressed as micromoles / 24 hr.

**ESTIMATION OF MAGNESIUM**

**Standard** : Magnesium metal 1 g of magnesium metal was dissolved in 50 ml of 5 N HCl and diluted to 1 litre with deionised water to give a standard containing 1000 µg of magnesium/ml.

<table>
<thead>
<tr>
<th>Light source</th>
<th>Hollow cathode lamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamp current</td>
<td>3.0 mA</td>
</tr>
<tr>
<td>Flame type</td>
<td>Air acetylene</td>
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</table>

![Table: Magnesium Estimation Parameters](image)

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Slit width (nm)</th>
<th>Working range (µg/ml)</th>
<th>Sensitivity (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>285.2</td>
<td>0.5</td>
<td>0.1-0.4</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Magnesium levels are expressed as micromoles/24hr.
RESULTS AND DISCUSSION

The present study demonstrates that the consequence of decreased glomerular filtration rate, reflected by increased urinary excretion of enzymes of both brush-border membrane and of lysosomal origin, and phospholipids are an early manifestation of gentamicin induced proximal tubular cell injury.

Table 1 represents the excretion pattern of urinary constituent in control and experimental groups.

Decreased kidney weight to body weight ratio was observed in the highest toxic group. The systemic toxicity of gentamicin has been reported from long term studies, shows that the kidney is the major target organ of gentamicin toxicity (Maldonado et al., 2003).

Urinary excretion of creatinine (p<0.001) and urea (p<0.001) were found to be significantly reduced during gentamicin treatment (Group III), when compared to that of control (Group I). This decreased excretion might be due to the decreased glomerular filtration rate (Espandiari et al., 2007; Ghaznaviet al., 2005). At high dose of gentamicin, reduction in glomerular plasma flow rate also plays a role. This observation suggests that alteration in glomerular filtration is one of the earliest manifestations of gentamicin nephrotoxicity. Administration of Morindacitrifolia extract to the nephrotoxic rats (Group IV) normalized the excretion pattern of creatinine and urea.

Proteinuria of glomerular and/or tubular origin is a routine index of nephrotoxicity which in chronic aminoglycoside nephrotoxicity appears to indicate mostly tubular proteinuria (Table 1, p<0.001). Proteinuria has also been described after treatment with gold (Chen et al., 2006), mercury (Escribeseetal., 2007), cadmium (Prozialecket al., 2007) and in the urine of patients with renal calculus disease. A similar pattern of proteinuria was observed after administration of adriamycin (Tao et al., 2008). The observed proteinuria in the present study (Group III) suggests that gentamicin-induced glomerular damage, and increased glomerular permeability for proteins and its catabolites. Gentamicin administration causes a definite tubular proteinuria, the excretion of proteins of molecular weight below 40,000, mainly β-2 macroglobulin, retinol-binding protein (RBP), lysozyme (LZM), immunoglobulin light chains and post-gamma globulins (Barataet al., 2001).
Tugay et al. (2006), also observed increased glomerular permeability to macromolecules during gentamicin treatment with reference to albumin, which supports our present observation.

Observed proteinuria during gentamicin therapy, was reverted to near normal by administration of *Morindacitrifolia* extract, which suggests nephroprotectant role (Group IV, p<0.001).

Gentamicin, causes inhibition of phopholipase C, accompanied by changes in phospholipids composition in the kidney cortex. There is increasing concern about secondary hyperlipidemia and changes in lipoproteins fraction which develops with drug-induced nephrotoxicity. Alterations or defects in the regulation of cholesterol metabolism have a profound effect on the cellular metabolism (Katz et al., 2007). Ackerman et al (2006), have shown an altered plasma lipid metabolism during gentamicin therapy.

**TABLE 1  URINARY EXCRETION PATTERN OF BIOCHEMICAL CONSTITUENTS IN CONTROL AND EXPERIMENTAL GROUPS**  
(Values are mean ± SD for six animals in each group)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I C</th>
<th>Group II C + MC</th>
<th>Group III GM</th>
<th>Group IV GM + MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney to body weight ratio</td>
<td>132.8 ± 21.4</td>
<td>128.1 ± 12.5</td>
<td>90.1 ± 7.4</td>
<td>110.0 ± 2.3</td>
</tr>
<tr>
<td>Creatinine (mg/24 hr)</td>
<td>7.01 ± 0.32</td>
<td>5.85 ± 0.12</td>
<td>1.81 ± 0.18&lt;sup&gt;a8&lt;/sup&gt;</td>
<td>4.54 ± 1.78&lt;sup&gt;bs&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein (mg/24 hr)</td>
<td>2.33 ± 0.56</td>
<td>2.00 ± 0.75</td>
<td>8.94 ± 1.71&lt;sup&gt;a8&lt;/sup&gt;</td>
<td>3.08 ± 0.06&lt;sup&gt;bs&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea (mg/24 hr)</td>
<td>10.61 ± 0.75</td>
<td>10.00 ± 0.98&lt;sup&gt;a8&lt;/sup&gt;</td>
<td>3.11 ± 1.20&lt;sup&gt;a8&lt;/sup&gt;</td>
<td>8.12 ± 0.14&lt;sup&gt;bs&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phospholipids (mg/24 hr)</td>
<td>128.1 ± 12.3</td>
<td>125.7 ± 15.3</td>
<td>157.25 ± 3.32&lt;sup&gt;a8&lt;/sup&gt;</td>
<td>99.9 ± 9.2&lt;sup&gt;bs&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

C-Control, MC-*Morindacitrifolia*, GM-Gentamicin.

Treatments of groups are as follows : Group I - Control,  
Group II - Control + *Morindacitrifolia*  
(500 mg/kg bw),  
Group III - Gentamicin (100 mg/kg bw),  
Group IV - Gentamicin + *Morindacitrifolia*.

Comparisions made between the groups are as follow :  
a - as compared with control  
b - as compared with gentamicin treated rats
The symbols represent statistical significance: * $p<0.05$, £ $p<0.01$, $^\$ p$<0.001$.

**Changes in Urinary Electrolyte Excretion Pattern**

Table 2 represents the changes in urinary excretion of electrolytes - calcium, magnesium, sodium, potassium and phosphorus in control and treatment groups.

**TABLE 2. URINARY EXCRETION OF ELECTROLYTES IN CONTROL AND EXPERIMENTAL ANIMALS**

(Values are mean ± SD for six animals in each group)

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Group I C</th>
<th>Group II C + MC</th>
<th>Group III GM</th>
<th>Group IV GM + MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (µmoles/24 hr)</td>
<td>9.62 ± 3.35</td>
<td>7.25 ± 2.82</td>
<td>14.63 ± 1.94£</td>
<td>12.25 ± 2.83</td>
</tr>
<tr>
<td>Magnesium (µmoles/24 hr)</td>
<td>54.93 ± 4.94</td>
<td>± 46.33 ± 7.21</td>
<td>60.23 ± 6.69</td>
<td>48.32 ± 2.00$^$</td>
</tr>
<tr>
<td>Sodium (mmoles/24 hr)</td>
<td>2.44 ± 0.13</td>
<td>2.48 ± 0.52</td>
<td>3.03 ± 0.41$^*$</td>
<td>2.75 ± 0.24$^*$</td>
</tr>
<tr>
<td>Potassium (mmoles/24 hr)</td>
<td>4.23 ± 0.16</td>
<td>4.51 ± 0.38</td>
<td>4.67 ± 0.23</td>
<td>4.08 ± 0.13</td>
</tr>
<tr>
<td>Phosphorous (mg/ 24 hr)</td>
<td>6.31 ± 0.24</td>
<td>7.37 ± 0.07$^$</td>
<td>8.73 ± 0.61$^$</td>
<td>6.22 ± 0.49$^$</td>
</tr>
</tbody>
</table>

C-Control, MC-*Morinda citrifolia*, GM-Gentamicin.

Treatments of groups are as in Table 1

Comparisons made between the groups are as in Table 1.

The symbols represent statistical significance: * $p<0.05$, £ $p<0.01$, $^\$ p$<0.001$

**FIGURE 2 URINARY EXCRETION OF ELECTROLYTES IN CONTROL AND EXPERIMENTAL ANIMALS**
Hypercalciuria, enhanced urinary magnesium output and phosphaturia are hall mark of gentamicin-induced nephrotoxicity (Garland et al., 1992). Displacement of calcium from tubular binding sites by gentamicin, or alterations in calcium regulating hormone, such as parathyroid hormone or a direct effect on transepithelial calcium transport by gentamicin might be the reasons for the observed calciuria. Calcium loading is shown to reduce gentamicin – induced nephrotoxicity by a suppression of PTH secretion (Bennett et al., 1985). Gentamicin has been shown to inhibit [Na⁺,K⁺]-ATPase (Fukuda et al., 1991), which in turn, has a bearing on calcium, because, sodium-dependent calcium reabsorption has been shown to be depressed under such circumstances.

**CONCLUSION**

Gentamicin administration decreased the excretion of creatinine and urea, with increased protein, calcium, magnesium, sodium, potassium and phosphorus, suggesting a loss in the reabsorptive capacity of renal proximal tubular cells. *Morindacitrifolia* administration reverted the urinary excretion of these biochemical constituents, to near normal suggesting a nephroprotectant role. Gentamicin administration caused increased level of phospholipids (p<0.05) (Table 1). Drug - induced phospholipidosis and lysosomal trapping of phospholipids – drug complexes in the kidney, caused a reduction in the circulating phospholipids. *Morindacitrifolia* administration to the gentamicin – induced nephrotoxic rats, reverted the lipid ionic changes to near normal. The urinary wasting of magnesium may be due to the decreased kidney and plasma magnesium concentration. Increased urinary excretion of Na⁺ and Mg⁺ was observed during gentamicin administration, and further its protection by the natural antioxidant curcumin supports our present result obtained with *Morindacitrifolia* (Group IV). Sodium and potassium showed no significant changes during
gentamicin administration. Gentamicin - induced tubular electrolytes wasting is likely to be a result rather than a cause of proximal tubular injury.

REFERENCES


