Assessment of gentamicin-induced nephrotoxicity in rats treated with low doses of ibuprofen and diclofenac sodium

M. M. FARAG, M. MIKHAIL, R. SHEHATA, E. ABDEL-MEGUID and S. ABDEL-TAWAB

Medical Research Institute and Faculty of Medicine, Alexandria University, Alexandria, Egypt

(Received 8 November 1995/23 February 1996; accepted 26 March 1996)

1. The effects of two non-steroidal anti-inflammatory drugs, ibuprofen (20 mg day\(^{-1}\) kg\(^{-1}\)) and diclofenac sodium (2.5 mg day\(^{-1}\) kg\(^{-1}\)), on the severity of gentamicin-induced nephrotoxicity were evaluated in rats.

2. Administration of gentamicin (100 mg day\(^{-1}\) kg\(^{-1}\)) for 5 days resulted in a significant increase in renal cortical total phospholipids accompanied by a significant decrease in cortical Na\(^{+}\), K\(^{+}\)-ATPase activity. These changes were associated with a significant decrease in body weight and increases in kidney weight, serum creatinine and urea nitrogen.

3. In rats treated simultaneously with both gentamicin and either ibuprofen or diclofenac sodium for 5 days, all the measured parameters of renal dysfunction were similar in magnitude to those observed in rats treated with gentamicin alone.

4. In contrast, rats treated with either ibuprofen or diclofenac sodium for 27 days and injected concurrently with gentamicin during the last 5 days of the treatment period had significantly higher kidney weight, lower renal cortical Na\(^{+}\), K\(^{+}\)-ATPase activity and higher cortical phospholipid content, serum creatinine and urea nitrogen than did rats treated with gentamicin alone. A 27-day treatment with ibuprofen or diclofenac sodium alone resulted in no change in renal function.

5. These results demonstrate that gentamicin nephrotoxicity was potentiated after the long (27 days) but not after the short (5 days) period of treatment with ibuprofen and diclofenac sodium. Thus, prolonged administration of non-steroidal anti-inflammatory drugs should be considered as a risk factor that may increase the nephrotoxic potential of gentamicin.

INTRODUCTION

Gentamicin, a widely used aminoglycoside antibiotic, is recognized to possess significant nephrotoxic potential in man and experimental animals [1-4]. Gentamicin-induced renal injury is almost exclusively confined to proximal tubular cells, where the drug selectively accumulates [5, 6]. Within these cells, gentamicin induces an early accumulation of phospholipids accompanied by a reduction in the activity of Na\(^{+}\), K\(^{+}\)-ATPase, the major regulator of the cation gradients across plasma cell membranes [6-8]. Late manifestations of gentamicin nephrotoxicity include inhibition of proximal tubular transport processes, depression of glomerular filtration rate and proximal tubular cell necrosis [9].

Several studies have presented evidence that prostaglandin (PG) production is increased in the kidneys of gentamicin-treated animals [10-12]. This response is believed to be a compensatory mechanism for maintaining renal blood flow and glomerular filtration rate within normal or near-normal limits during the development of gentamicin nephrotoxicity [11, 13, 14]. As the cyclo-oxygenase enzyme is involved in the synthesis of renal prostaglandins [14], the objective of the present investigation was to evaluate the effects of two cyclo-oxygenase inhibitors, ibuprofen and diclofenac sodium, on the severity of gentamicin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Animals and treatments

Adult male albino rats of a local strain (Medical Research Institute, Alexandria, Egypt) weighing between 100 and 200 g were used. All rats were kept under observation for at least 1 week before study with free access to food and water. The rats were randomly divided into 12 groups and two experimental studies were carried out.

Study I. This assessed the effects of ibuprofen and diclofenac sodium on gentamicin nephrotoxicity in rats treated simultaneously with both drugs (ibuprofen plus gentamicin and diclofenac sodium plus gentamicin) for 5 days. The drug treatment of these groups was similar to that used during the last 5 days (from day 23 to day 27) of study II (Table 1).

Study II. This assessed the effects of ibuprofen and diclofenac sodium on gentamicin nephrotoxicity

Key words: aminoglycosides, diclofenac sodium, gentamicin, ibuprofen, nephrotoxicity, non-steroidal anti-inflammatory drugs.

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin.

Correspondence: Dr M. M. Farag, Department of Pharmacology, Medical Research Institute, Alexandria University, 165 El-Horria Avenue, El-Hadara, PO Box 21561, Alexandria, Egypt.
Table 1. Drug treatment in study II experimental groups.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>No. of rats</th>
<th>Drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days 1-22</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>Propylene glycol</td>
</tr>
<tr>
<td>Ibuprofen (I)</td>
<td>6</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Diclofenac sodium (D)</td>
<td>6</td>
<td>Diclofenac sodium</td>
</tr>
<tr>
<td>Gentamicin (G)</td>
<td>6</td>
<td>Propylene glycol</td>
</tr>
<tr>
<td>I+G</td>
<td>6</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>D+G</td>
<td>6</td>
<td>Diclofenac sodium</td>
</tr>
</tbody>
</table>

Table 2. Body weight (g) of control and drug-treated rats before the treatment periods of studies I and II. Values shown are means ± SEM of six or eight (control) rats. For treatment details, see Table 1. P-values compare day 6 and day 1 data (study I) or day 28 and day 23 data (study II) *P < 0.05, **P < 0.01, ***P < 0.001.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Study I (5-day treatment)</th>
<th>Study II (27-day treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On day 1</td>
<td>On day 6</td>
</tr>
<tr>
<td>Control</td>
<td>159 ± 13.1</td>
<td>167 ± 13.4***</td>
</tr>
<tr>
<td>Ibuprofen (I)</td>
<td>193 ± 14.4</td>
<td>203 ± 14.4***</td>
</tr>
<tr>
<td>Diclofenac sodium (D)</td>
<td>182 ± 14.4</td>
<td>191 ± 14.7***</td>
</tr>
<tr>
<td>Gentamicin (G)</td>
<td>174 ± 5.0</td>
<td>166 ± 7.0***</td>
</tr>
<tr>
<td>I+G</td>
<td>157 ± 12.8</td>
<td>148 ± 13.1*</td>
</tr>
<tr>
<td>D+G</td>
<td>168 ± 9.9</td>
<td>159 ± 9.0**</td>
</tr>
</tbody>
</table>

Epstein [18]. The method of Chen et al. [19] was used to measure inorganic phosphorus (Pi) concentration. The method of Lowry et al. [20] was used to determine protein concentration using bovine serum albumin as a standard. Na+, K+-ATPase activity was expressed in μg Pi h⁻¹ mg⁻¹ protein. Total phospholipids were extracted from another portion of the renal cortical tissue using the procedure of Folch et al. [21] and were quantitated using the Boehringer Mannheim kit for phospholipid assay (Mannheim, Germany). The renal cortical total phospholipid content was expressed in mg/g tissue. The blood samples obtained from the rats were allowed to clot and centrifuged. The separated sera were used for measuring serum creatinine and urea nitrogen [22].

Statistical analysis
The results are presented as means ± SEM. For statistical comparison among the experimental groups, analysis of variance (one-way classification) and Duncan's multiple range test with Kramer's adjustment for unequal sample sizes were used [23]. The paired t-test was used for comparing the body weight data in each experimental group. Values of P < 0.05 were considered to represent significant differences between means.

RESULTS
As shown in Table 2, control, ibuprofen-treated and diclofenac sodium-treated rats exhibited an

Procedures
The animals were killed by decapitation and the blood from each rat was collected in a centrifuge tube. The kidneys were immediately removed and weighed and the renal cortex was rapidly dissected free. A portion of this cortical tissue was assayed for Na+, K+-ATPase activity as described by Katz and
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Fig. 1. Kidney weight expressed as percentage of body weight in control (C) and drug-treated (I, ibuprofen; D, diclofenac sodium; G, gentamicin) rats after 5 days of treatment. Each bar represents the mean ± SEM of six or eight (C) rats. ∗P < 0.01 compared with the C, I and D groups. Unless otherwise indicated, differences between groups are not significant.

Fig. 2. Kidney weight expressed as percentage of body weight in control (C) and drug-treated (I, ibuprofen; D, diclofenac sodium; G, gentamicin) rats in study II. For treatment details, see Table I. Each bar represents the mean ± SEM of six or eight (C) rats. ∗P < 0.01 as compared with the C, I and D groups. †P < 0.05 as compared with the G alone group.

increase in body weight during the treatment periods of studies I and II (5 and 27 days respectively). In both studies, when gentamicin (100 mg day⁻¹ kg⁻¹) was administered for 5 days alone and concomitantly with either ibuprofen or diclofenac sodium, a significant decrease in body weight was evident in all treated rats. In each experimental study, when the percentages of weight loss were compared, no significant differences were observed between the group treated with gentamicin alone and the groups treated with the combined therapy.

Figures 1 and 2 illustrate the effects of drug therapy on kidney weight. In all gentamicin-treated rats, a significant increase in kidney weight (expressed as percentage of body weight) was observed (P < 0.01). In the 5-day treatment study, the increase in kidney weight was similar in magnitude whether gentamicin was given alone or in combination with either ibuprofen or diclofenac sodium. In the 27-day treatment study, kidney weight increased by 40.7%, 67.2% and 62.9% of the corresponding control values in rats treated with gentamicin, ibuprofen plus gentamicin and diclofenac sodium plus gentamicin, respectively, during the last 5 days of the treatment period (P < 0.01). Thus, in both groups treated with the combined therapy after pretreatment with the two NSAIDs, the increase in kidney weight was more pronounced than that seen in rats treated with gentamicin alone (P < 0.05).

The results presented in Table 3 demonstrate that treatment with gentamicin for 5 days caused a decrease in renal cortical Na⁺, K⁺-ATPase and increases in renal cortical total phospholipid content, serum creatinine and urea nitrogen (P < 0.01). No significant differences were detected with respect to these parameters among the three groups treated with gentamicin (alone and in combination with either ibuprofen or diclofenac sodium) in the 5-day treatment study. The longer duration of NSAID therapy (27 days), however, had evident effects on the renal changes caused by gentamicin. As summarized in Table 4, all gentamicin-treated groups, in the second set of experiments, showed evidence of nephrotoxicity as indicated by the significant changes in the measured metabolic and biochemical parameters as compared with the results from the control animals (P < 0.01). In these experiments, animals treated with either ibuprofen or diclofenac sodium for 27 days and injected concurrently with gentamicin during the last 5 days of the treatment period had significantly lower renal cortical Na⁺, K⁺-ATPase activity and higher cortical phospholipid, serum creatinine and urea nitrogen levels than rats treated with the vehicle (propylene glycol) and gentamicin (P < 0.05). As shown in Tables 3 and 4, treatment with either ibuprofen or diclofenac sodium alone did not significantly alter the renal cortical and serum parameters as the measured levels were within the control limits.

DISCUSSION

The nephrotoxicity of aminoglycoside antibiotics, and especially of the most commonly used compound, gentamicin, is well documented [2, 24, 25]. As expected, all rats injected with gentamicin for 5 days at a dose of 100 mg day⁻¹ kg⁻¹ showed nephrotoxic effects. In agreement with previous studies reported by other investigators [6–8], gentamicin administration, in the present study, produced renal cortical phospholipidosis and a decrease in renal cortical Na⁺, K⁺-ATPase activity. These changes were associated with a decrease in rat body weight and increase in kidney weight in comparison with controls. All these findings were consistent with the observed alteration in renal function as reflected by a significant increase in both serum creatinine and urea nitrogen as compared with control values. This
Table 3. Effects of 5-day treatment with ibuprofen (I), diclofenac sodium (D) and gentamicin (G) on renal cortical Na⁺, K⁺-ATPase activity, total phospholipid (TP) content, serum creatinine and urea nitrogen. Values shown are means ± SEM of six or eight (control) rats. *Significantly different from control, I and D groups (P<0.01). Unless otherwise indicated, differences between groups are not significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>I</th>
<th>D</th>
<th>G</th>
<th>I+G</th>
<th>D+G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, K⁺-ATPase (µg P, h⁻¹ mg⁻¹ protein)</td>
<td>4.43 ± 0.30</td>
<td>4.96 ± 0.18</td>
<td>4.33 ± 0.27</td>
<td>2.23 ± 0.20*</td>
<td>2.34 ± 0.17*</td>
<td>2.56 ± 0.19*</td>
</tr>
<tr>
<td>TP content (mg/g tissue)</td>
<td>31.29 ± 0.64</td>
<td>30.67 ± 0.99</td>
<td>30.70 ± 1.05</td>
<td>38.29 ± 1.15*</td>
<td>40.35 ± 0.90*</td>
<td>38.76 ± 0.66*</td>
</tr>
<tr>
<td>Serum creatinine (mg/100 ml)</td>
<td>0.66 ± 0.05</td>
<td>0.68 ± 0.04</td>
<td>0.65 ± 0.05</td>
<td>1.85 ± 0.22*</td>
<td>1.56 ± 0.15*</td>
<td>1.89 ± 0.17*</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/100 ml)</td>
<td>15.50 ± 1.71</td>
<td>13.62 ± 0.77</td>
<td>11.94 ± 1.56</td>
<td>47.47 ± 4.85*</td>
<td>42.87 ± 4.18*</td>
<td>49.27 ± 4.31*</td>
</tr>
</tbody>
</table>

Table 4. Effects of 5-day treatment with gentamicin (G) on renal cortical Na⁺, K⁺-ATPase activity, total phospholipid (TP) content, serum creatinine and urea nitrogen in rats treated with ibuprofen (I) and diclofenac sodium (D) for 27 days. Values shown are means ± SEM of six or eight (control) rats. For treatment details, see Table 1. *Significantly different from control, I and D groups (P<0.01). †Significantly different from the G alone group (P<0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>I</th>
<th>D</th>
<th>G</th>
<th>I+G</th>
<th>D+G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, K⁺-ATPase (µg P, h⁻¹ mg⁻¹ protein)</td>
<td>4.64 ± 0.28</td>
<td>4.84 ± 0.29</td>
<td>4.95 ± 0.34</td>
<td>2.40 ± 0.23*</td>
<td>1.55 ± 0.12†</td>
<td>1.37 ± 0.11†</td>
</tr>
<tr>
<td>TP content (mg/g tissue)</td>
<td>31.21 ± 0.75</td>
<td>31.54 ± 0.80</td>
<td>31.49 ± 0.64</td>
<td>38.00 ± 1.16*</td>
<td>44.39 ± 1.94†</td>
<td>48.27 ± 1.98*</td>
</tr>
<tr>
<td>Serum creatinine (mg/100 ml)</td>
<td>0.72 ± 0.03</td>
<td>0.67 ± 0.06</td>
<td>0.62 ± 0.06</td>
<td>1.56 ± 0.21†</td>
<td>2.56 ± 0.29†</td>
<td>2.72 ± 0.30†</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/100 ml)</td>
<td>15.34 ± 1.71</td>
<td>14.27 ± 2.27</td>
<td>15.10 ± 1.62</td>
<td>42.25 ± 7.12*</td>
<td>86.13 ± 9.33†</td>
<td>92.15 ± 12.82†</td>
</tr>
</tbody>
</table>

elevation in serum parameters, in gentamicin-treated rats, was probably the result of tubular necrosis with a consequent decrease in the number of functioning nephrons [26, 27].

The results of the first series of experiments indicate that concomitant administration of ibuprofen or diclofenac sodium with gentamicin for 5 days did not affect the severity of nephrotoxicity induced by the latter agent. This conclusion is in agreement with the findings of Assael et al. [11], who observed no effect of acetylsalicylic acid (100 and 200 mg/day kg⁻¹) on gentamicin nephrotoxicity after 5 days of the combined therapy in rats. In the present study, the effects of a longer period (27 days) of NSAID treatment on the nephrotoxicity of gentamicin (100 mg/day kg⁻¹ for 5 days) were also evaluated. The results of these experiments clearly indicate that concomitant administration of ibuprofen or diclofenac sodium with gentamicin caused a considerable increase in gentamicin nephrotoxicity in the rats pretreated with either of the two NSAIDs. In these experiments, the increase in kidney weight, the decrease in renal cortical Na⁺, K⁺-ATPase activity and the increase in renal cortical phospholipid content, serum creatinine and urea levels were more marked in rats treated with the combined therapy than in rats treated with gentamicin alone. It is also evident from the data of the present investigation that a 27-day treatment with ibuprofen or diclofenac sodium alone had no effect on the kidney as determined by the measured renal cortical metabolic and serum parameters. Thus, the increase in gentamicin nephrotoxicity observed in rats treated for 27 days with ibuprofen or diclofenac sodium was not caused by an additive toxic effect of the two drugs (gentamicin and ibuprofen or gentamicin and diclofenac sodium) on the kidney.

As ibuprofen and diclofenac sodium act within the cyclooxygenase pathway of arachidonic acid metabolism [17], the present results may best be explained by the presence of a relationship between prostaglandins and renal haemodynamics. Prostaglandin E₂ and PGI₂ are potent renal vasodilators and have been detected in high concentrations in the kidney [14, 28]. These prostaglandins, however, do not appear to alter renal haemodynamics significantly under normal conditions [14]. Several studies have demonstrated that renal parenchymal disease resulting from different causes, including nephrotoxic agents such as aminoglycosides, represents a situation in which active prostaglandin production may maximize the glomerular filtration rate and renal blood flow in surviving nephrons [10, 11, 14, 29, 30]. In this case, NSAID therapy may cause impairment of renal haemodynamics by inhibiting renal prostaglandin biosynthesis and, thereby, wor-
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sen renal insufficiency [14, 30, 31]. It should be emphasized that the NSAID-induced renal haemodynamic changes are more prominent with prolonged therapy and in the presence of renal injury [30, 31]. Therefore, it is probable that the NSAID-induced ischaemic renal insult resulting from inhibited prostaglandin production in the kidney could be, at least in part, responsible for the present results with respect to the observed potentiation of gentamicin nephrotoxicity in rats treated with ibuprofen and diclofenac sodium for 27 days.

In conclusion, the present investigation demonstrates that NSAIDs such as ibuprofen and diclofenac sodium, in doses close to those used in human therapy, may enhance gentamicin nephrotoxicity. The duration of the NSAID therapy before commencement of gentamicin administration appears to be an important factor in the NSAID–gentamicin interaction. Although it is difficult to extrapolate from animal studies to clinical medicine, our investigation with two NSAIDs in doses close to the therapeutic ones strongly suggests that the present findings may have pertinence for humans as well. Thus, in view of the increasing popularity of NSAIDs and the wide clinical use of gentamicin, the possibility of concomitant administration of a NSAID with gentamicin with a subsequent increase in the nephrotoxic potential of the latter agent should be considered.

REFERENCES