Protective Effect of Urinary Alkalization on Gentamicin Nephrotoxicity in Rats

Ho Yung Lee,1 Kyu Hun Choi1 and Norman Bank2

We investigated the effect of urinary alkalization accomplished by intraperitoneal injection of sodium bicarbonate and acetazolamide on gentamicin nephrotoxicity in male Fisher 344 rats. Forty rats (body weight 200-300g) were divided into four groups: control (gentamicin 20mg/kg, bid), high sodium load (gentamicin 20mg/kg, 25cc of saline, bid), low bicarbonate (gentamicin 20mg/kg, 25cc of sodium bicarbonate 100mEq/L, 2.5mg of Diamox, bid) and high bicarbonate (gentamicin 20mg/kg, 10cc of sodium bicarbonate 250mEq/L, 2.5mg of Diamox, bid) groups. All drugs and electrolyte solutions as mentioned above were administered intraperitoneally twice a day for seven days and changes in renal functions were studied. While salt loading failed to influence the severity of gentamicin nephrotoxicity, urinary alkalization induced by bicarbonate and acetazolamide injection showed remarkable ameliorating effects on gentamicin nephrotoxicity. The high bicarbonate group exhibited more beneficial effects than the low bicarbonate group on gentamicin nephrotoxicity. So, urinary alkalization seems to be an effective method for the prevention of gentamicin nephrotoxicity in rats.

Key Words: Urinary alkalization, gentamicin nephrotoxicity.

For the past 20 years, aminoglycoside antibiotics such as gentamicin, kanamycin and tobramycin have been extensively used in the treatment of gram-negative infections. In more recent years, their application in combination with penicillins or cephalosporins has grown steadily in gram positive infections, taking advantage in some cases of antibacterial synergism (Appeal et al. 1978). However, despite such usefulness, aminoglycosides have a major limiting factor, nephrotoxic acute renal failure, which has continued to concern clinicians (Appeal et al. 1978; Bennett 1983). Many studies on the factors potentiating the nephrotoxicity resulting from gentamicin, have been performed (Adelman et al. 1979; Bennett et al. 1976; Chiu et al. 1979; Elliott et al. 1980).

From those studies, a number of risk factors such as volume depletion (Lecompte et al. 1981), metabolic acidosis (Hsu et al. 1974) hypokalemia (Dobyan et al. 1982), advancing age and pre-existing renal insufficiency (Moore et al. 1984) were defined. So, although most experiments were carried out using animal models, it was recommended that gentamicin be used more carefully in patients with these risk factors. In addition, several attempts to reduce gentamicin nephrotoxicity, such as the administration of sodium bicarbonate or a high calcium diet have also been made (Bennett et al. 1976; Bennett et al. 1982; Chiu et al. 1979; Lecompte et al. 1981), but according to the authors, the results were somewhat contradictory.

Regarding the effect of urinary alkalization among these interventions, Chiu et al. (1978) observed a reduction in nephrotoxicity and in the renal cortical concentration of gentamicin in rats given sodium bicarbonate and gentamicin together. In another report, no significant improvement in gentamicin nephrotoxicity was seen in animals drinking sodium bicarbonate (Elliott et al. 1980). However, since there is much evidence indicating that the attachment of gentamicin to the brush border membrane in proximal tubular cells is electrostatic in nature (Bennett 1983; Collier et al. 1979; Kluwe and Hook 1978), urinary alkalization is considered as a possible way to interfere with the binding of gentamicin and its ameliorating nephrotoxicity. Therefore, the following study was undertaken to investigate the effect of urinary
alkalinization on the prevention of gentamicin nephrotoxicity in male Fischer 344 rats.

MATERIAL AND METHODS

Adult male Fischer 344 rats weighing 200-300g were selected, placed in individual metabolic cages and randomly divided into 4 groups of 10 rats each, as follows:

Group 1 (Control): Animals were injected with gentamicin intraperitoneally 20 mg/kg twice a day for 7 consecutive days.

Group 2 (High sodium load group): Rats received intraperitoneal injection of gentamicin 20 mg/kg and 0.9% NaCl solution 25 ml² twice a day for 7 days.

Group 3 (Low bicarbonate group): Animals were injected intraperitoneally with gentamicin 20 mg/kg, 25 ml² of NaHCO₃ 100 mEq/L and 2.5 mg of Diamox twice a day for 7 days.

Group 4 (High bicarbonate group): Rats received intraperitoneal injection of gentamicin 20 mg/kg, 10 ml² of NaHCO₃ 250 mEq/L and 2.5 mg of Diamox twice a day for 7 days.

All animals were allowed tap water ad libitum and fed a sodium free diet. After 3 to 4 days of adaptation to the diet, blood samples were obtained by cardiac puncture while the animals were under light ether anesthesia, and analyzed for blood urea nitrogen (BUN), serum creatinine (Biochemica Test Combination, Boehringer Manheim, GMBH, 1972), sodium, potassium (Coleman flame photometer, model 51) and CO₂ content on the day prior to initiating gentamicin and on the next day of the last injections. 24 hours urine samples were also collected under oil in the metabolic cages on the same day as obtaining blood. The concentrations of creatinine and sodium in the urine and the volume of the 24 hour urine sample were measured.

Creatinine clearance was calculated by the standard method. Fractional excretion of sodium was defined as the ratio of clearance of sodium to clearance of creatinine. The clearance of sodium was calculated as \( \frac{U_{Na} \times V}{P_{Na}} \), where \( U_{Na} \) and \( P_{Na} \) represent urine and plasma sodium concentration, respectively.

Data were expressed as the mean ±S.D. The student’s t-test was employed for statistical analysis, and a p value of <0.05 was defined as significant.

RESULTS

There was no significant difference in the pretreatment values of BUN, serum creatinine and creatinine clearance among the four groups, and the administration of gentamicin for 7 days caused the deterioration of renal function in all four groups. But as shown in Table 1, the degree of deterioration in each group was not identical.

Changes in BUN level and serum creatinine concentration

The level of BUN on the last day of the gentamicin injection period increased significantly above the

Table 1. Changes in renal function after 7 days of drug administration

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>Scr (mg/dl)</th>
<th>Ccr (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D7-0</td>
<td>D7-7</td>
<td>D0</td>
</tr>
<tr>
<td>Control</td>
<td>mean</td>
<td>14.17</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>4.07</td>
<td>125.78</td>
</tr>
<tr>
<td>High Na</td>
<td>mean</td>
<td>12.00</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>4.65</td>
<td>12.49</td>
</tr>
<tr>
<td>Low HCO₃</td>
<td>mean</td>
<td>12.60</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2.07</td>
<td>13.16</td>
</tr>
<tr>
<td>High HCO₃</td>
<td>mean</td>
<td>9.20</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>1.92</td>
<td>9.59</td>
</tr>
</tbody>
</table>

D7: days after drug injection.
* p<0.05 when compared with the control
BUN: Blood urea nitrogen
Scr: Serum creatinine concentration
Ccr: Creatinine clearance

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pretreatment value in all four groups (Table 1, Fig. 1). The increase in the control group was much greater than that in either the low or high bicarbonate group \( (p<0.05, \text{Table 1}) \), whereas it was not statistically different from that in the high sodium load group \( (p>0.05, \text{Table 1}) \). There was no significant difference in the level of BUN between the low and high bicarbonate groups.

Similar to the data for BUN, serum creatinine concentration became highly elevated after gentamicin treatment in the control, high sodium load and low bicarbonate group \( (p<0.05, \text{Table 1, Fig. 2}) \). The serum creatinine concentration of the control group did not also differ much from that of the high sodium load group \( (p>0.05, \text{Table 1}) \), but it was significantly higher than that of the low bicarbonate group \( (\text{Table 1}) \). In the high bicarbonate group, although the difference was not statistically significant, serum creatinine concentration rather decreased slightly below the pretreatment concentration. So it was far lower than that of the control or low bicarbonate group \( (p<0.05, \text{Table 1}) \).

These results suggest that urinary alkalization with bicarbonate preserves renal function adequately during the gentamicin injection periods, but sodium load has no protective effect against the gentamicin induced acute renal failure in rats. The preventive effect on gentamicin nephrotoxicity appear to be more beneficial with the use of bicarbonate solution of high concentration compared to that of low concentration.

Changes in creatinine clearance and urine output

The administration of gentamicin for 7 days caused a significant decrease in creatinine clearance in all 4 groups \( (\text{Table 1, Fig. 3}) \). While the decrease in the control group was markedly greater than that in the low or high bicarbonate groups \( (p<0.05, \text{Table 1}) \), there was no statistical difference between the control and the high sodium load groups \( (p>0.05, \text{Table 1}) \). Animals in the high bicarbonate group exhibited a much higher creatinine clearance than did the low bicarbonate group after gentamicin administration \( (p<0.05) \).

In the high sodium load, low bicarbonate and high

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**Fig. 1.** BUN levels before and after 7 days of drug injections. Abbreviations are: C, Control group; Na, High sodium load group; LB, Low bicarbonate group; HB, High bicarbonate group.

**Fig. 2.** Serum creatinine levels before and after 7 days of drug injections. Abbreviations as in Fig. 1.
bicarbonate groups, urine output measured before treatment with gentamicin was significantly greater than that in the control group, and polyuria in these three groups was thought to be induced by the administration of fluids in large amounts (Table 2). After gentamicin injection, urine output was markedly reduced in all groups except the low bicarbonate group (p<0.05, Table 2), and as illustrated in Figure 4, the reduction of urine output in the high sodium load group seemed to be largest. But there was no significant difference in the 24 hour urine volume after treatment with gentamicin among all groups except

Fig. 3. Creatinine clearance (Ccr) before and after 7 days of injections. Abbreviations as in Fig. 1.

Fig. 4. Relative urine volume to control (V/C x 100) after 7 days of drug injections. Abbreviations as in Fig. 1.

| Table 2. Changes in laboratory findings before and after 7 days of drug administration |
|------------------------------------------|----------|----------|----------|----------|----------|
| Group     | V (ml/d) | U_{eq} (mM/L) | U_{eq}V (mM/d) | FENA (%) |
|           | D-7      | D-0      | D-7      | D-0      | D-7      |
| Control   | mean     | 10.02    | 4.00     | 7.68     | 0.25     | 0.11     | 0.21     | 0.04     | 23.49   |
| High Na   | mean     | 32.90*   | 2.13     | 17.47*   | 0.20     | 0.55*    | 0.0004   | 1.13*    | 26.10   |
|           | S.D.     | 7.78     | 0.00     | 8.01     | 0.00     | 0.21     | 0.00     | 0.39     | 0.00    |
| Low HCO_3 | mean     | 33.34*   | 45.46*   | 42.62*   | 70.20*   | 1.42*    | 3.14*    | 0.31*    | 2.59*   |
|           | S.D.     | 3.22     | 6.91     | 6.70     | 19.81    | 0.27     | 0.82     | 0.14     | 1.99    |
| High HCO_3| mean     | 24.12*   | 7.16     | 76.44*   | 79.70*   | 1.83*    | 0.61*    | 0.46*    | 0.75*   |
|           | S.D.     | 5.35     | 3.05     | 17.38    | 18.08    | 0.56     | 0.06     | 0.25     | 0.27    |

*p<0.05 when compared to the control
V: Urine volume per day
D+: days after drug injection
U_{eq}: Urine CO_2 concentration
U_{eq}V: Total amount of 24 hour urine bicarbonate
FENA: Fractional Na excretion
the low bicarbonate group (Table 2). In the low bicarbonate group, urine output rather slightly increased, though it did not reach a significant level (p>0.05). Therefore, 24 hour urine volume after gentamicin injection in the low bicarbonate group was significantly different from that of the other three groups (Table 2).

Changes in the concentration and amount of urinary CO$_2$

The pretreatment concentration of urinary CO$_2$, which was measured as an index of urinary alkalization in our experiment, was much higher in the high sodium load, low bicarbonate and high bicarbonate groups than in the control group (p<0.05, Table 2). Since the reabsorptive capacity for bicarbonate varied directly with the fractional reabsorption of sodium, the cause of bicarbonaturia in the high sodium load group appeared to be the decreased reabsorption of sodium under the state of volume expansion. After gentamicin injection for 7 days, a significant fall in urinary CO$_2$ concentration was observed in the control and high sodium load groups, whereas urinary CO$_2$ concentration in both bicarbonate groups remained high (Table 2). The urinary CO$_2$ concentration in the control group was statistically much lower than that of the low or high bicarbonate groups (p<0.05, Table 2, Fig. 5). The rats in the high sodium load group did not exhibit any statistical difference in urinary CO$_2$ concentration from the control group (p>0.05, Table 2). As with the concentration of urinary CO$_2$, the amount of urinary CO$_2$ before treatment with gentamicin was significantly larger in the high sodium load, low bicarbonate and high bicarbonate groups than in the control group. However, on the next day after 7 days of the gentamicin injection period, the amount of urinary CO$_2$ reflecting urinary bicarbonate excretion remained high in both low and high bicarbonate groups whereas those in control and high sodium load groups are negligible (Table 2).

Changes in fractional excretion of sodium (FENa)

The fractional excretion of sodium on the day prior to the initial gentamicin injection was far lower in the control group than the other three groups (p<0.05, Table 2), and animals in the high sodium load group exhibited the highest value. This was due to the reduced tubular reabsorption of sodium which was attributed to the high sodium content in the fluid medium. After 7 days of gentamicin injections, fractional excretion of sodium was greatly increased in

![Fig. 5. Urine CO$_2$ content after 7 days of drug injections. Abbreviations as in Fig. 1.](image)

![Fig. 6. Fractional Na excretion (FENa) before and after 7 days of injections. Abbreviations as in Fig. 1.](image)
all groups above the pretreatment values (p<0.05, Table 2, Fig. 6), but that of the low or high bicarbonate groups maintained at very low level and was statistically much lower than that of the control and high sodium load groups (p<0.05, Table 2). While the high bicarbonate group showed a fractional excretion of sodium which was significantly lower than that of the low bicarbonate group, the difference in fractional excretion of sodium between the control and the high sodium load group was not significant.

**DISCUSSION**

It is well known that the administration of the aminoglycoside antibiotic gentamicin frequently results in the development of nephrotoxic acute renal failure in humans and experimental animals (Appel and Neu 1978; Moore et al. 1984). This form of acute renal failure is manifested histologically as proximal tubular necrosis, and ultrastructurally, increased number of large irregular dense lysosomes which contain myeloid bodies are found in the damaged proximal tubular cells (Houghton et al. 1976). In rats receiving gentamicin, the renal cortical concentration of gentamicin is many times the plasma level (Lecompte et al. 1981), and Collier et al. (1979) observed luminal uptake of gentamicin in the isolated perfused rat kidney. Therefore it is reasonable to assume a correlation between the uptake of gentamicin in the proximal tubular cells and the resultant nephrotoxicity. Recently, concern has been raised that the cortical accumulation might be linked to the potential nephrotoxicity of gentamicin.

The mechanism by which gentamicin is accessed to and retained by proximal tubular cells is unclear, but biochemical and autoradiographic studies by Morin et al. (1980) suggest that gentamicin accumulates in the proximal tubular cell by endocytosis and lysosomal sequestration, being preceded by binding to the brush border membrane. According to Sastrasinh et al. (1982), the binding appears to take place between cationic polybasic gentamicin and anionic acidic phospholipids in the brush border membrane. Elliott et al. (1980) reported that the avidity of luminal binding of gentamicin was correlated with the number of free amino groups which were the cationic portions of molecule. So the binding of gentamicin to the brush border membrane seems to be electrostatic in nature.

Gentamicin is a mixture of three closely related congeners, C1, C2, and C3 which have 5 amino groups each and 5 corresponding pKa's varying between 6.3 and 9.6 (Scholtan and Rosenkranz 1978). According to Elliott et al. (1980), the pH at which half of the amino groups of gentamicin are charged is 8.4, whereas at pH 7.4, 90% of the amino groups are charged. It was also reported that while gentamicin accumulation at alkaline pH under oxygen was similar to that at pH 7.4, accumulation at acidic pH was significantly greater than that at pH 7.4 (Kluwe and Hook 1978). In this regard, altering the cationic charges of the gentamicin molecule may influence the binding and subsequent epithelial uptake of gentamicin byys changed affinity for the luminal membrane.

In male Sprague-Dawley rats, Chiu et al. (1979) found that renal uptake of gentamicin was moderately inhibited by the intravenous infusion of sodium bicarbonate with ameliorating nephrotoxicity. On the other hand, Elliott et al. (1980) reported that male Fischer 344 rats drinking water containing sodium bicarbonate did not show a significant difference in serum creatinine concentration or renal cortical concentration of gentamicin, compared with those drinking tap water. The reasons for these different results are not clear, but the difference in the strain of rat or administration route of the sodium bicarbonate is suggested. Our experiment was carried out on male Fisher 344 rats, which was the same strain of rat used in study by Elliott et al. (1980), and all drugs and electrolyte solutions were injected intraperitoneally in order to more precisely control the administration of them.

Concerning the changes in renal functions after a 7 day injection period, rats given gentamicin in combination with sodium bicarbonate and acetazolamide maintained renal functions better than those receiving gentamicin only, and this finding was in conflict with the descriptions by Elliott et al. (1980). As the same strain of rat was used in our experiment this discrepancy might be derived mainly from the different administration route of bicarbonate. In addition, animals in the high bicarbonate group exhibited a much lower serum creatinine concentration and higher creatinine clearance than the low bicarbonate group. Therefore, in our study, urinary alkalization accomplished by administration of sodium bicarbonate and acetazolamide offered a significant protective effect from gentamicin nephrotoxicity, and the administration of sodium bicarbonate in a high concentration appeared to be more effective in reducing nephrotoxicity. In view of the hypothesis described above, this protective effect of urinary alkalization seems to be mediated by inhibiting the binding of gentamicin to the brush border membrane of proximal tubule. However, since it is not confirmatory
whether there is a quantitative relationship between the renal cortical concentration of gentamicin and the degree of nephrotoxicity, further studies are needed.

Meanwhile, it has been reported that casts containing cellular debris were formed in many tubules injured by gentamicin (Houghton et al. 1976; Wellwood et al. 1976) and from micropuncture experiment, Neugarten et al. (1983) asserted that varying degrees of tubular obstruction contributed to the reduction of the glomerular filtration rate in acute renal failure due to gentamicin. This evidence supports the view that intratubular obstruction by cast plays a role in gentamicin nephrotoxicity. So, considering that the Tamm-Horsfall protein which constitutes the matrix of the cast has greater solubility in an alkaline solution (McQueen 1962), it seems likely that urinary alkanization achieved as the present study was effective in reducing intratubular obstruction.

In the high sodium load group, renal function after treatment with gentamicin for 7 days was no significantly different from the control group. Bennett et al. (1976) demonstrated that despite reduction of renal cortical gentamicin concentration, serum creatinine concentrations in rats with high sodium intake were similar to those in normally fed rats. So, the finding in the high sodium load group was in agreement with the result of Bennett et al. (1976), though the renal cortical concentration of gentamicin was not measured in our experiment. It has also been reported that rehydration did not decreased the plasma and renal cortical concentration of gentamicin in dehydrated rats treated with gentamicin (Lecompte et al. 1981). Therefore, while volume depletion was believed to be one of the risk factors which increased susceptibility to the toxicity of gentamicin, a high load of sodium in a large amount of fluid was not effective in alleviating the nephrotoxicity caused by gentamicin. Mild bicarbonaturia in the high sodium load group appeared not to have a significant effect on modifying gentamicin nephrotoxicity.

After administration of gentamicin for 7 days, the fractional excretion of sodium which expressed the fraction of filtered sodium escaping reabsorption, was more elevated in the control group than in the low or high bicarbonate group, and the high sodium load group did not show a significant difference in the fractional excretion of sodium from the control group. The increased fractional excretion of sodium observed in patients with acute renal failure was consistent with the reduced proximal tubular sodium reabsorption (Hsu et al. 1978). So, the most likely explanation for increased fractional excretion of sodium in the present study was thought to be decreased reabsorption of sodium in the proximal tubules damaged by gentamicin, though the influence of volume expansion in either the high sodium or low bicarbonate group could not be completely excluded. Hence, in the case of fractional excretion of sodium, gentamicin-induced renal insults on the proximal tubule seemed to be also less severe in both the low and high bicarbonate groups than in the control or high sodium load groups.

From these results, it may be concluded that urinary alkalinization is a very effective method for the prevention of gentamicin nephrotoxicity in rats and further study may be needed to clarify any similar ameliorating effect on gentamicin nephrotoxicity by urinary alkalinization in humans.

References

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