

## BIOCHEMICAL ROLE OF ELECTROLYTE EXCRETION IN AMINOGLYCOSIDE NEPHROTOXICITY

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### ABSTRACT

**Objective:** To access the role of electrolyte excretion in aminoglycoside renal damage using experimental rats. **Methods:** Six groups each comprising five glycerol-pretreated and non-glycerol-pretreated rats were employed. In the later, Group 1 served as control and received normal saline (1 ml/kg body weight) intraperitoneally (ip). Groups II, III, IV, V and VI received Netilmicin, gentamycin, frusemide, netilmicin + gentamycin, and netilmicin + frusemide ip respectively. While in the former (glycerols-pretreated), Groups II, III, IV, V and VI received netilmicin, gentamycin, frusemide, netilmicin + gentamycin and netilmicin +gentamycin + frusemide ip respectively. Electrolytes were determine using EEL Model 920 chloride meter and EEL flame photometer for alkali metal ions in the urine samples. Reproducible data were analyzed with a digital Vax/Vms computer using Monitab programme for the one-way analysis of variance and student T-test. **Results:** in the non-glycerol pretreated animals, only those that received gentamicin excreted significantly ( $p < 0.05$ ) more urinary chloride, caused a slight increase in urinary potassium excretion, and increased the urinary excretion of sodium than the control. While in glycerol-pretreated animals, only those that received netilmicin + gentamycin showed similar urinary chloride excretion to the control but a marked increase in the urinary excretion of potassium in those that received netilmicin alone; and netilmicin produced a decrease (unlike gentamicin) in urinary sodium excretion. **Conclusion:** Renal excretion of electrolytes indicate progressive renal damage, yet its sensitivity was not high enough based on the large standard of error, as a biochemical parameter for measuring aminoglycoside nephrotoxicity.

**KEYWORDS:** nephrotoxicity, aminoglycosides, electrolyte excretion, biochemical role, gentamicin, netilmicin, frusemide.

### INTRODUCTION

The two most important of all the organs and glands of excretion are the kidneys and lungs.<sup>[1]</sup> The functional unit of the kidney remains the nephron. The two kidneys together accounts for less than 1% of the total body mass yet they receive nearly 25% of the cardiac output. Consequent upon this, the kidney cells are constantly exposed to more chemical insult than are the cells of most other organs.<sup>[2]</sup> The various microvilli at the luminal border highly increase reabsorptive area as the proximal tubule is ideally structured for the massive salt and water reabsorption. Water and chloride (Cl<sup>-</sup>) are reabsorbed by massive diffusion along osmotic gradients. About 55 to 80% of the postglomerular filtrate is reabsorbed before the tubular fluid passed into the loop of Henle. The fluid has as its major ionic constituents, sodium and chloride. As the hyper-osmotic urine from the loop of Henle gets to the collecting duct, hydrogen (H<sup>+</sup>) and potassium (K<sup>+</sup>) ions and ammonia (NH<sub>3</sub>) passively enter the tubular fluid. Potassium secretion can

be accompanied by active reabsorption of sodium (Na<sup>+</sup>), chloride, bicarbonate (HCO<sub>3</sub><sup>-</sup>) and potassium ions out of the lumen and back into the interstitial fluids.<sup>[3]</sup> The fact that vertebrate liver can modify molecules by conjugating them with glucuronic acid or sulphate for secretion via active transport mechanisms located in the wall of the nephron, innumerable substance including H<sup>+</sup> and K<sup>+</sup> ions, ammonia, organic acids and organic bases can be secreted by the nephron.<sup>[4]</sup> Preservation of kidney function is an important chemical goal, hence several measures have been employed to understand the integrity of the mammalian kidney. For instance, the ideal measurement of glomerular filtration rate is insulin clearance but the text is technically complicated and is rarely applied in clinical studies. Other methods, in view of this difficulty, such as turbidity, urinary excretion of electrolytes and renal histology have been employed to study renal function.<sup>[5]</sup>

Aminoglycosides are eliminated mainly unmetabolised via the kidney. The large renal blood flow results in high delivery of blood-bone toxicants to the kidney as compared with other organs of excretion. The sodium/Potassium ( $\text{Na}^+/\text{K}^+$ ) pump is bound to the tubular cell membrane regulating intercellular  $\text{Na}^+$  concentration and by excretion, intracellular fluids volume, similar to that induced by ischaemic damage, nephrotoxicants frequently inactivate the  $\text{Na}^+/\text{K}^+$  pump, hence causing cell swelling and death. Toxicants those are either secreted or reabsorbed by tubular epithelial cell. Gentamicin (a toxicants) and other aminoglycosides are commonly known to cause hospital-acquired acute renal failure.<sup>[6]</sup> Gentamicin is known to be more nephrotoxic than netilmicin in patients with healthy kidneys<sup>[7]</sup>. Earlier researchers have shown also that nephrotoxicity induced by gentamicin is much less severe in the pre-nephrotic patients than in patients with healthy kidney.<sup>[8]</sup> The present investigation was undertaken to estimate the magnitude of electrolyte losses in aminoglycoside-induced nephrotoxicity and possibly determine the sensitivity of renal electrolyte excretion as a biochemical parameter to measure aminoglycoside-induced nephrotoxicity.

## MATERIALS AND METHODS

### Non-glycerol pretreated rats

Adult rats weighing 200 to 350 g were used. The animals were in six groups of 5 rats each, as in the following protocol:

- Group I received normal saline 1 ml/kg body weight, ip
- Group II received Netilmicin 100 mg/kg body weight, ip
- Group III received gentamicin 100 mg/kg body weight, ip
- Group IV received frusemide 50 mg/kg body weight, ip
- Group V received netilmicin + gentamicin, ip
- Group VI received netilmicin + gentamicin + frusemide, ip.

### Glycerol-pretreated rats

Adult rats weighing 200 to 350 g were used. The animals were in six groups of 5 rats each, as in the following protocol:

- Group I received 50% glycerol in normal saline 4 ml/kg body weight, ip
- Group II received Netilmicin 100 g/kg body weight
- Group III received gentamicin 100 mg/kg body weight, ip
- Group IV received frusemide 50 mg/kg body weight, ip
- Group V received Netilmicin + gentamicin
- Group VI received Netilmicin + gentamicin + Frusemide

The animals in all protocols were kept in separate glass metabolism cages and were allowed free access to

standard rat food and water. Twenty four (24) hour urine samples were collected, the volume measured and the electrolytes were determined.

### Measurement of electrolytes

#### Chloride ( $\text{Cl}^-$ ) ion.

The "EEL" Model 920 Chloride meter was used to determine urinary chloride concentrations.

#### Potassium ( $\text{K}^+$ ) and Sodium ( $\text{Na}^+$ ) ions

The "EEL" flame photometer was employed to measure the relative concentrations of alkali metal ions in the urine samples. The instrument was calibrated with a series of KCL solutions with a concentration range of 0.02 to 1.0 mg per 100 ml with respect to sodium ion.

### Statistical analysis

Reproducible data collected from the experimentations were statistically analysed with a Digital Vax/Vms computer using Minitab Programme for the one way analysis of variance and student T-Test. Mean and SEM is significant when  $P < 0.05$ .

## RESULTS

### Urinary Chloride ( $\text{Cl}^-$ )

In the non-glycerol pretreated rats, animals that received gentamicin only excreted significantly ( $P < 0.05$ ) urinary chloride more than the control in 24 h (Figure 1). Netilmicin showed lower urinary excretion of chloride than the control, as did frusemide. The concomitant administration of netilmicin and gentamicin showed that chloride excretion was half the control value and when the two aminoglycosides were administered together with frusemide, the urinary excretion of chloride was slightly less than that of the control (Figure 1). In the glycerol-pretreated animals, only those that received netilmicin plus gentamicin had similar urinary chloride excretion rates when compared with the control. Other groups that received either gentamicin, or netilmicin, or frusemide alone or in various combinations excreted significantly less chloride compared with the control.

### Urinary Potassium ( $\text{K}^+$ )

In the non-glycerol pretreated rats, animals that received gentamicin only caused a slight increase in urinary potassium excretion compared with the control value. Concomitant administration of netilmicin and gentamicin however, caused a marked decrease in potassium excretion as did frusemide with the combined administration of netilmicin plus gentamicin with frusemide (Figure 3). There was a marked rise in the urinary excretion of potassium in the glycerol-pretreated rats that received netilmicin. This effect did not change appreciably when frusemide was administered concomitantly with netilmicin. Netilmicin plus Frusemide however, decreased the renal excretion of potassium more than did gentamicin with frusemide which of course, values did not vary significantly from the control value (Figure 4). Even the concomitant administration of the two aminoglycosides plus

frusemide, did not significantly decrease the urinary excretion of potassium.

**Urinary Sodium (Na<sup>+</sup>)**

In the non-glycerol pretreated animals, gentamicin markedly increased the excretion of urinary sodium while netilmicin decreased it. The concomitant administration of both aminoglycosides significantly ( $P < 0.05$ ) inhibited renal sodium excretion than when both were concomitantly administered plus frusemide.

However, combined administration of either gentamicin with frusemide or netilmicin with frusemide, significantly ( $P < 0.05$ ) decreased the excretion of urinary sodium (Figure 5). In the glycerol-pretreated rats, netilmicine also produced a decrease while gentamicin showed an increase in urinary sodium excretion. In comparison with the non-glycerol pretreated animals, the concomitant administration of netilmicin and gentamicin showed a significant increase in urinary sodium excretion in the glycerol-pretreated rats (Figure 6).

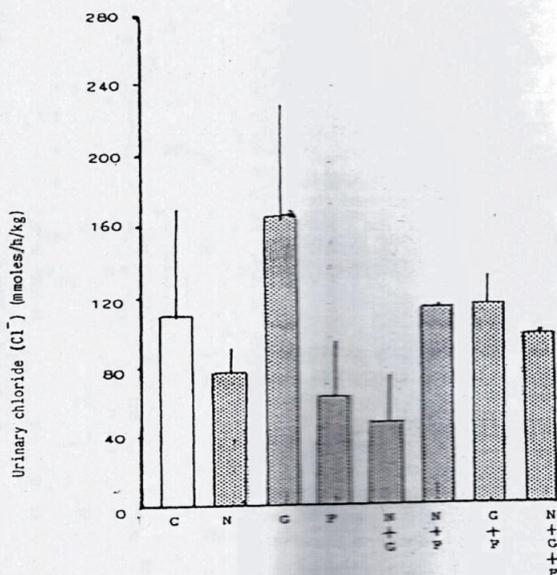


Figure 1 Chloride ion excretion in non-glycerol pretreated rat 48 hours after I.P. injection of Netilmicin, Gentamicin and Frusemide alone and in combination.

C = Control  
 G = Gentamicin (100 mg/kg)  
 N = Netilmicin (100 mg/kg)  
 F = Frusemide (50 mg/kg)  
 Each bar represents values from 5 animals

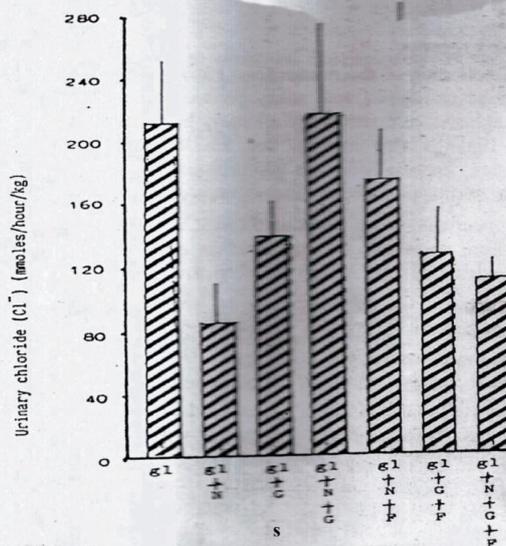
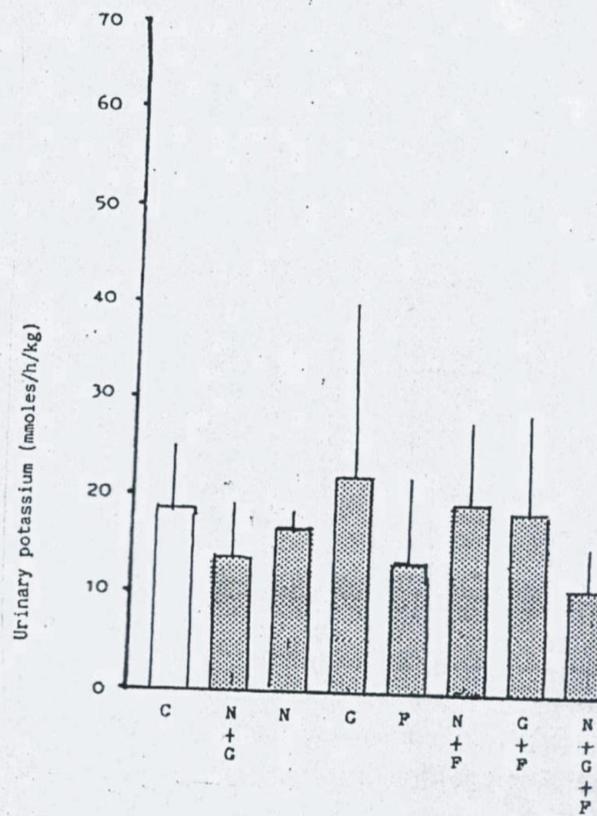
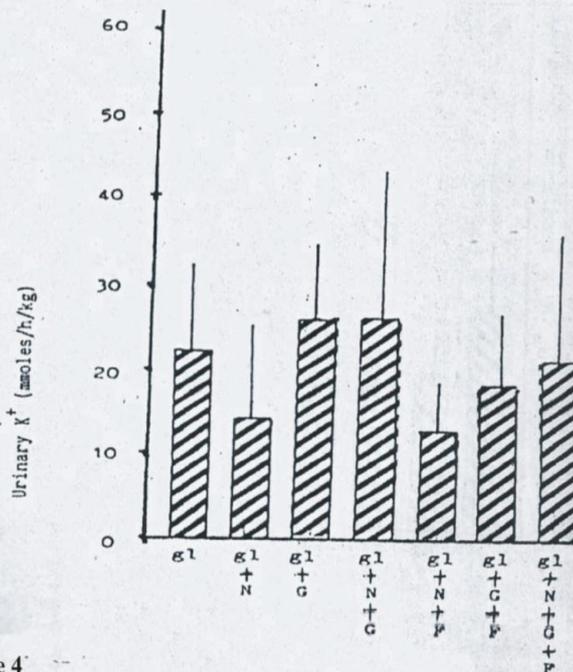


Figure 2 Chloride ion excretion in non-glycerol pretreated rat 48 hours after I.P. injection of Netilmicin, Gentamicin and Frusemide alone and in combination.

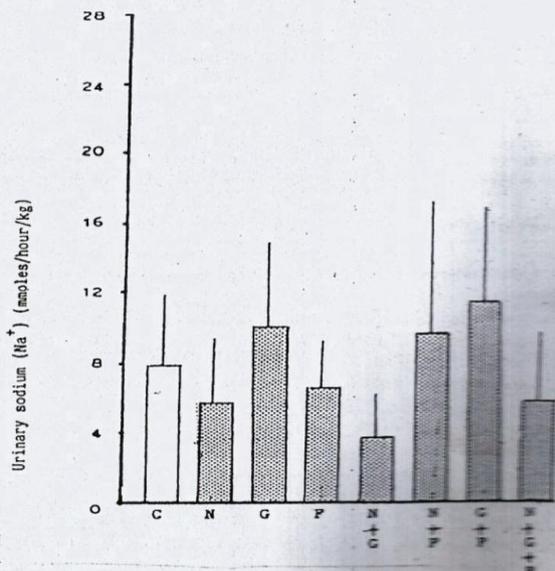


**Figure 3**  
Potassium ion excretion in non-glycerol-pretreated rats 48 hours after i.p. injection of Netilmicin, Gentamicin and Frusemide alone or in combination

C = Control  
 G = Gentamicin (100 mg/kg)  
 N = Netilmicin (100 mg/kg)  
 F = Frusemide (50 mg/kg)  
 Each bar represents values from 5 animals

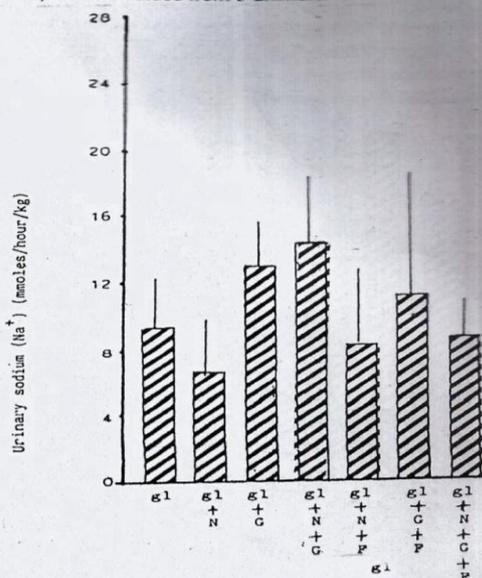


**Figure 4**  
Potassium ion excretion in glycerol-pretreated rats 48 hours after i.p. injection of Netilmicin, Gentamicin and Frusemide alone or in combination



**Figure 5**  
Sodium ion excretion in non-glycerol pretreated rat 48 hours after I.P. injection of Netilmicin, Gentamicin and Frusemide alone or combination.

C = Control      G = Gentamicin (100mg/kg)  
N = Netilmicin (100/kg)      F = Frusemide (50 mg/kg)  
Each bar represents values from 5 animals.



**Figure 6**  
Sodium ion excretion in glycerol-pretreated rats 48 hours after i.p. injection of Netilmicin, Gentamicin and Frusemide alone or in combination

**DISCUSSIONS**

The result showed that aminoglycoside-induced renal damage was associated with urinary electrolyte losses especially in the glycerol-pretreated animals. This is in consonance with documented report that a significant rise in serum potassium, severe renal dysfunction, and severe widespread histological changes of acute tubular necrosis were linked to combination therapy of gentamicin with ketorolac.<sup>[9]</sup> Gentamicin clearance has been shown to be decreased in dogs with subclinical renal dysfunction, pre-existing renal disorder and advanced age often associated with some degree of decreased renal function, can increased the potential by several mechanisms for nephrotoxicity.<sup>[10]</sup> The result of this study also showed that gentamicin administration

was associated with increased urinary excretion of potassium and consequently increased nephrotoxicity. This is in agreement with documented studies in dogs that dietary potassium restriction exacerbates gentamicin nephrotoxicity, probably because potassium-depleted cells are susceptible to necrosis.<sup>[11]</sup> Gentamicin-induced nephrotoxicity is much less severe in pre-nephrotic patients than in patients with healthy kidney.<sup>[8]</sup> The findings in this study with experimental rats corresponds with a report that netilmicin raises the urinary excretion of sodium and potassium to a lesser extent than gentamicin.<sup>[12]</sup> and concomitant administration of gentamicin and frusemide enhances the nephrotoxicity of gentamicine.<sup>[13,1]</sup> Frusemide possibly potentiates gentamicin-induced nephrotoxicity by causing a degree

of dehydration hence reducing the volume of distribution of gentamicin and increasing its renal tubular absorption. It should be noted that dehydration and volume depletion are perhaps, the most common and important risk factors for acute renal failure. In human beings, studies indicate that volume depletion increases the patient's risk of developing acute renal failure by a factor of ten.<sup>[14]</sup>

## CONCLUSION

Electrolyte loss is an index of aminoglycoside-induced renal damage however, exaggerated standard error of mean (SEM) values was observed, associated with these urinary excretion of electrolytes, hence it is not a sensitive biochemical parameter for determining early renal damage in animal studies, and by extension possibly in human.

## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

## SOURCE OF SUPPORT

Nil.

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