

## MALNUTRITION AS AN ADDITIONAL RISK FACTOR TO GENTAMICIN NEPHROTOXICITY

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### ABSTRACT

The association between malnutrition and infectious diseases has long been described. Gentamicin is an aminoglycoside antibiotic that is widely used for the treatment of severe gram-negative infections, despite its nephrotoxicity. Clinical and experimental studies have also shown important alterations in renal function during malnutrition. In this way, the aim of the present study was to verify if the use of gentamicin (G) in rats subjected to food restriction (R) could interfere with the development of gentamicin nephrotoxicity. Two month-old Male Wistar rats were submitted to food restriction (50%) during 30 days. In the last 10 days, they were treated with saline or gentamicin (40 mg/kg/day ip). The studied groups were: C)- rats with ad libitum food intake + saline, G)- ad libitum food intake + gentamicin, R)- food-restricted + saline, RG)- food-restricted + gentamicin. A significant fall in glomerular filtration rate (GFR) was observed in groups R ( $5.69 \pm 0.22$ ) and G ( $5.31 \pm 0.27$ ) when compared to group C ( $7.17 \pm 0.42 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ). In RG group, the impairment in GFR was more evident than in groups G or R, ( $4.42 \pm 0.24 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ). In all the experimental groups, the decrease in GFR occurred in parallel to the decrease in renal plasma flow (RPF) so that the filtration fraction (FF%) was maintained. A decline in urine/plasma inulin ratio was observed in both groups treated with gentamicin and also in food restricted group. Although G and R "per se" caused an increased excretion of  $\text{Na}^+$ , the highest values were obtained in RG group. Although our study has been performed in an experimental model, if extrapolated to human protocols, it can be suggested that the use of aminoglycoside antibiotics in malnourished patients should be performed with caution, specially concerning renal function and considering the potential increased risk exhibited by these patients.

**Key words:** kidney; gentamicin, nephrotoxicity; rats; malnutrition; renal function  
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### INTRODUCTION

In undeveloped countries, malnutrition (MN) is still the most important public-health problem which underscores the high rates of morbidity and mortality. The prevalence of MN, according to the World Health Organization (WHO), is more than 30% of the world's infant population (1). MN is usually the reflex of synergistic factors such as low family income, low educational levels and poor environment

and housing conditions, which facilitate the contact with infectious agents. In fact, the association between MN and infectious diseases has long been described in the history (2). Impairment in both humoral and cellular immunity present in cases of malnutrition can aggravate the prognostic in face of infectious episodes.

Gentamicin is an aminoglycoside antibiotic that has been widely used in the treatment of infections caused by gram-negative bacteria, due to its ef-

ficacy and low cost (3). The antibacterial activity is mediated by an irreversible inhibition of the bacteria protein synthesis at 30S ribosomal level (4). Gentamicin is excreted by glomerular filtration and is partially reabsorbed by renal proximal tubules. The tubules also accumulate the antibiotic and are the primary site of nephrotoxicity which is an important limiting factor in its clinical use (5,6). Several clinical and experimental studies have been performed concerning drug dose, patient's age, and administration schedule, in order to investigate factors that can aggravate or attenuate aminoglycoside nephrotoxicity. Among these factors, different therapeutic regimens are being increasingly explored because of the recent interest in the once-a-day schedule, whose current evidence suggests to be as safe and effective as multiple daily doses (7-9).

Few studies have investigated the possible influence of nutritional status on the development of aminoglycoside nephrotoxicity. Since aminoglycoside therapy is frequently indicated to malnourished patients, this study was designed to investigate the influence of nutritional status on the development of gentamicin nephrotoxicity.

## MATERIALS AND METHODS

Male Wistar rats weighing around 200 g were obtained from Paulista School of Medicine, São Paulo, Brazil. They were housed individually in plastic cages with sawdust on the floor and maintained inside a temperature controlled room ( $25^{\circ}\text{C} \pm 0.5$ ) with dark-light cycle of 12:12 hours (lights on at 8:00 am). Water was provided ad libitum. The animals received care in accordance with the guidelines of the institutional review board and animal use committees.

The animals were divided into 4 groups. During 30 days, 2 groups received ad libitum food intake, while the other 2 groups received only 50% of the intake recorded from the ad libitum groups. During the last 10 days (from the 21<sup>st</sup> until 30<sup>th</sup>) of the experiment, gentamicin (40 mg/kg) or saline was injected intraperitoneally in the animals once a day. The following groups were, thus, studied: C)- Control, with ad libitum food intake, that received saline, G)- ad libitum food intake that received gentamicin, R)-

Food-restricted rats, that received saline, RG)- Food-restricted that received gentamicin.

On the day 31<sup>st</sup>, animals were anesthetized with sodium thiopental 100 mg/kg (ip) and prepared for clearance measurements. In brief, the jugular vein, carotid artery and urinary bladder were cannulated for infusions and blood or urine withdrawal, respectively. Tracheostomy was also performed.

Clearance measurements - The glomerular filtration rate (GFR) was evaluated by the inulin clearance (10) and the renal plasma flow (RPF) by the sodium p-aminohippurate (PAH) clearance (11). Animals were primed with 1 ml of saline containing inulin (90 mg) and sodium p-aminohippurate (PAH; 2 mg) and then submitted to a continuous infusion of a solution containing inulin (15 mg/ml) and PAH (4 mg/ml) at a rate of 0.05 ml/min. After a stabilization period of 30 min, 4 clearance periods of 30 min were obtained. Blood samples were drawn at the midpoint of each clearance period. Urine was collected quantitatively in pre-weighed containers, and urine volume was determined gravimetrically. Inulin and PAH concentration in plasma and urine were measured colorimetrically (10,11). The inulin and PAH clearances (Cl) were calculated using the following formula:  $\text{Cl} = \text{U}/\text{P} \cdot \text{V}$ , where U and P are the concentration of inulin or PAH in urine and plasma samples respectively, and V is the urinary flow. U/P inulin ratio is also used as an index of urinary concentration capacity. Filtration fraction (FF%) was calculated using the formula:  $\text{GFR}/\text{RPF} \cdot 100$ .

Blood and urinary  $\text{Na}^+$  and  $\text{K}^+$  were measured using an  $\text{Na}^+ / \text{K}^+$  analyzer, model 248 (Ciba-Corning Diagnostics Ltd., Essex, England). Fractional excretion (FE%) of sodium, potassium or bicarbonate were calculated using the formula:  $\text{EA}/\text{FA} \cdot 100$ , where: EA is the excreted amount and FA is the filtrated amount. Urine and blood pH,  $\text{pCO}_2$  and bicarbonate values were obtained with a Ciba-Corning, model 614 blood gas analyzer.

Data are means  $\pm$  standard error (SE); n is the number of measurements. Differences between experimental groups were evaluated by analysis of variance followed by the Tuckey test or Kruskal Wallis test when appropriate. For all analysis the null hypothesis rejection level was set at 0.05.

## RESULTS

At the end of the experimental period, the animals subjected to food restriction presented, as expected, a lower body weight ( $214 \pm 3.19$  g) than that observed in animals with ad libitum food intake ( $296 \pm 5.62$  g),  $p < 0.05$ . All the groups presented values of acid-base status in the normal range.

The parameters of renal homodynamic are shown in Table-1. In R and G groups, a similar fall in GFR, RPF and U/P inulin ratio were observed, when compared to those from control values. When both, food restriction and gentamicin treatment were present as in RG, GFR and U/P decreased even more, suggesting that food restriction can worsen the nephrotoxicity of gentamicin.

Values for fractional excretion (FE%) of sodium, potassium and bicarbonate are shown in Table-2. Food restriction and gentamicin treatment caused a similar increase in FE%Na ( $1.22 \pm 0.09$  for R and  $1.44 \pm 0.14$  for G, vs.  $0.46 \pm 0.07$  in C,  $p < 0.05$ ). Gentamicin treatment led to a significant increase in FE%K, when compared to values from C group,  $30.38 \pm 2.62$  vs.  $20.78 \pm 1.38\%$  ( $p < 0.05$ ), respectively. When both conditions were present as in RG group, the increase in FE%K was more accentuated, even compared to G, ( $49.53 \pm 3.60$  vs.  $30.38 \pm 2.62\%$ ,  $p < 0.05$ ). Although gentamicin treatment tended to increase the FE%HCO<sub>3</sub> in control rats, food restriction led to a significant impairment in tubular reabsorp-

tion of bicarbonate as shown by the increased values for this parameter in both R and RG groups when compared to C or G ( $p < 0.05$ ).

## DISCUSSION

Nephrotoxicity is an important factor which can limit the use of aminoglycosides. Among them, gentamicin, has been widely used, due to its efficacy and low cost (3). In a recent study by Caksen et al., (12) it was shown that most part of urinary tract infections were caused by *Escherichia coli*, whose strains were resistant to co-trimoxazole (82.3%), ceftriaxone (17.6%), cefotaxime (17.6%), and ciprofloxacin (17.6%); none of the studied strains were resistant to gentamicin, reinforcing the importance of this kind of antibiotic in the treatment of gram-negative infections.

In the present work, it was observed that gentamicin affected, more intensely, the glomerular function, the urinary concentration ability and also the fractional excretion of potassium in the group submitted to food restriction than the observed in the ad libitum group. Previous work from this laboratory showed that rats treated with several types of aminoglycosides exhibited a significant decrease in GFR, RPF and in sodium and potassium tubular reabsorption, (5,6). On the other hand, clinical and experimental studies of MN have shown changes in renal functional parameters such as impairment in re-

**Table 1** - Parameters of renal homodynamic in groups: Control (C), Gentamicin (G), Food-restricted (R), and Food-restricted + Gentamicin (RG). The results are reported as mean  $\pm$  SE. \*  $P < 0.05$  vs. C; § vs. G; ‡ vs. R. The number of measurements was 24, and the number of animals was 6, in each group.

Group	GFR ml.min <sup>-1</sup> .kg <sup>-1</sup>	RPF ml.min <sup>-1</sup> .kg <sup>-1</sup>	FF%	U/P inulin	V ml.min <sup>-1</sup> .kg <sup>-1</sup>
C	$7.17 \pm 0.42$	$19.97 \pm 1.87$	$35.75 \pm 0.48$	$52.93 \pm 2.09$	$0.133 \pm 0.005$
R	$5.69 \pm 0.22^*$	$15.80 \pm 0.64^*$	$36.17 \pm 0.55$	$34.15 \pm 1.49^*$	$0.168 \pm 0.005^*$
G	$5.31 \pm 0.27^*$	$14.87 \pm 0.78^*$	$35.76 \pm 0.47$	$41.76 \pm 2.44^*$	$0.130 \pm 0.006$
RG	$4.42 \pm 0.24^*‡$	$13.32 \pm 0.66^*$	$33.24 \pm 0.92$	$24.97 \pm 1.27^*§‡$	$0.182 \pm 0.007^*§$

GFR: glomerular filtration rate; RPF: renal plasma flow; FF%: filtration fraction; U/P inulin: urine/plasma inulin ratio; V: urinary flow.

**Table 2** - Fractional excretion of sodium ( $FE\%Na^+$ ), potassium ( $FE\%K^+$ ), and bicarbonate ( $FE\%HCO_3^-$ ) in groups: Control (C), Gentamicin (G), Food-restricted (R), and Food-restricted + Gentamicin (RG). The results are reported as mean  $\pm$  SE. \*  $P < 0.05$  vs. C; § vs. G; ‡ vs. R. The number of measurements was 26, and the number of animals was 6 in each group.

Group	$FE\%Na^+$ $\mu eq.min^{-1}.kg^{-1}$	$FE\%K^+$ $\mu eq.min^{-1}.kg^{-1}$	$FE\%HCO_3^-$ $\mu eq.min^{-1}.kg^{-1}$
C	$0.46 \pm 0.07$	$20.78 \pm 1.38$	$0.076 \pm 0.006$
R	$1.22 \pm 0.09^*$	$20.91 \pm 0.80$	$0.232 \pm 0.046^*$
G	$1.44 \pm 0.14^*$	$30.38 \pm 2.62^*$	$0.136 \pm 0.027$
RG	$2.08 \pm 0.28^*$	$49.53 \pm 3.60^{*‡§}$	$0.261 \pm 0.021^{*§}$

nal homodynamic, concentration ability, and acid excretion (13,14). Klahr et al. (14) studied the effects of malnutrition on renal concentrating ability and suggested that a diminished medullary urea concentration could reduce the effective tubular to interstitium gradient, leading to impairment in the concentration capacity. Another possibility could be the interference with the  $Na^+/K^+$  ATPase caused by malnutrition (15). Studies in children with protein-caloric malnutrition have consistently demonstrated reductions in GFR and effective renal plasma flow (ERPF), which were reverted after protein repletion (16,17). Micropuncture studies performed in protein deprived rats have shown that glomerular capillary ultrafiltration coefficient (Kf) was reduced in rats fed low protein diet; moreover, isolated glomeruli from low-protein-fed rats showed a smaller planar surface area than those from pair-fed isocaloric normal protein diets. Increased production of angiotensin II was held accountable for the decrease in glomerular filtration surface area, the fall in Kf, and the reduction in snGFR and whole kidney GFR (18). Changes in Kf, tubular  $Na^+/K^+$  ATPase and antidiuretic hormone (ADH) action were also described during gentamicin nephrotoxicity. (19,20). Our data show that the use of gentamicin impaired the reabsorption of  $Na^+$ , which could result in extracellular volume depletion and consequent stimulus to renin-angiotensin system. In food-restricted rats, a model that has already alterations in extracellular volume homeostasis, gentamicin treatment could impose an additional factor which could aggravate both glomerular and tubular function.

Bicarbonate excretion was observed to be increased in the groups submitted to food restriction, R and RG. Bicarbonate reabsorption in the proximal tubule is mediated mainly by  $Na^+/H^+$  antiporter localized in the brush border membrane (21). Studies by Harris et al. (22) have shown a decrease in the  $Na^+/H^+$  antiporter in renal brush border membrane vesicles from animals kept on a low protein diet. It is possible that this mechanism is responsible for the bicarbonate wasting observed in R and in RG. A tendency to elevate the bicarbonate excretion, although not significant, was observed in G; previous work from this laboratory showed that during metabolic acidosis, aminoglycoside treatment caused impairment in proximal bicarbonate reabsorption. However, the capacity of distal bicarbonate reabsorption was maintained (5,6). Our present data suggest that restricted animals are able to keep an adequate acid-base equilibrium even in the presence of gentamicin treatment, suggesting that the intact distal tubular function can compensate the impairment in proximal  $Na^+/H^+$  antiporter.

Although our study has been performed in an experimental model, if extrapolated to human protocols, the present results deserve some comments. The use of aminoglycoside antibiotics in malnourished patients is not unusual, since this condition is frequently seen in severely ill patients. In this way, the possibility of an enhancement of nephrotoxic effects must be taken into account, considering the potential increased risk that may be present in these circumstances.

## REFERENCES

1. Onis M, Monteiro C, Akre J, Clugston G: The worldwide magnitude of protein-energy malnutrition: an overview from the WHO global database on child growth. *Bull World Health Organ*, 71: 703-712, 1993.
2. Davidson S, Passmore R, Brock JF, Truswell, AS: Nutrition and Infection. In: *Human Nutrition and Dietetics*. Edinburg, Churchill Livingstone, 7th Ed, Chapt 59, p. 505, 1979.
3. Hitt CM, Klepser ME, Nightingale CH, Quintiliani R, Nicolau DP: Pharmacoeconomic impact of once-daily aminoglycoside administration, *Pharmacother*, 17: 810-814, 1997.
4. Kadurugamuwa JL, Clarke AJ, Beveridge TJ: Surface action of gentamicin on *Pseudomonas aeruginosa*. *J Bacteriol*, 175: 5798-5805, 1993.
5. Costa Silva VL, Gil FZ, Nascimento G, Cavanal MF: Evaluation of distal tubular function in aminoglycoside-induced nephropathy. *Braz J Med Biol Res*, 20: 833-836, 1987.
6. Silva VL, Gil FZ, Nascimento G, Cavanal MF: Effect of gentamicin on urinary acidification in the rat. *Ren Physiol*, 9: 204-212, 1986.
7. Gilbert DN, Lee BL, Dworkin RJ, Leggett JL, Chambers HF, Modin G et al.: A randomized comparison of the safety and efficacy of once-daily gentamicin or thrice-daily gentamicin in combination with ticarcillin-clavulanate. *Am J Med*, 105: 182-191, 1998.
8. Beauchamp D, Collin P, Grenier L, LeBrun M, Couture M, Thibault L et al.: Effects of fasting on temporal variations in nephrotoxicity of gentamicin in rats. *Antimicrob Agents Chemother*, 40: 670-676, 1996.
9. Uijtendaal EV, Rademaker CM, Schobben AF, Fleer A, Kramer WL, van Vught AJ: Once-daily versus multiple-daily gentamicin in infants and children. *Ther Drug Monit*, 23: 506-513, 2001.
10. Flores-Mendoza I, Mello Aires M, Malnic G: Effect of furosemide on urinary acidification during alterations of acid-base equilibrium in the rat. *J Bras Nefrol*, 1: 51-58, 1979.
11. Smith HW, Finkelstein N, Aliminosa L, Gralford B, Graber M: The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J Clin Invest*, 24: 388, 1945.
12. Caksen H, Cesur Y, Uner A, Arslan S, Sar S, Celebi V et al.: Urinary tract infection and antibiotic susceptibility. *Int Urol Nephrol*, 32: 245-247, 2000.
13. Benabe JE, Martinez Maldonado M: The impact of malnutrition on kidney function. *Miner Electrolyte Metab*, 24, 20-26, 1998.
14. Klarhr S, Tripathy K, Garcia FT, Mayoral LG, Ghitis J, Bolanos O: On the nature of the renal concentration defect in malnutrition. *Am J Med*, 43: 84-96, 1967.
15. Kudo LH, Shimizu MH, Seguro AC, Rocha AS: Renal concentrating defect in protein malnutrition: The role of thick ascending limb of Henle and inner medullary collecting duct. *Nephron*, 57: 156-163, 1991.
16. Gordillo G, Soto RA, Medcoff J, Lopez E, Antillon LG: Intracellular composition and homeostatic mechanisms in severe chronic infantile malnutrition. III Renal adjustments. *Pediatrics*, 20: 303-316, 1957.
17. Arroyave G, Wilson D, Behar M, Scrimshaw NS: Serum and urinary creatinine in children with severe protein malnutrition. *Am J Clin Nutr*, 9: 176-179, 1956.
18. Ichikawa I, Purkerson ML, Klahr S, Troy JL, Martinez-Maldonado M, Brenner BM: Mechanism of reduced glomerular filtration rate in chronic malnutrition. *J Clin Invest*, 65: 982-988, 1980.
19. Humes HD, Weinberg JM: The effect of gentamicin on antidiuretic hormone – stimulated osmotic water flow in the toad urinary bladder. *J Lab Clin Med*, 101: 472-478, 1983.
20. Fukuda Y, Malmberg AS, Aperia A: Gentamicin inhibition of Na<sup>+</sup>/K<sup>+</sup> ATPase in rat kidney cells. *Acta Physiol Scand*, 141: 27-34, 1991.
21. Preisig PA, Ives HE, Cragoe EJ Jr, Alpern RJ, Rector FC Jr: Role of the Na<sup>+</sup>/H<sup>+</sup> antiporter in rat proximal tubule bicarbonate absorption. *J Clin Invest*, 80: 970-978, 1987.

22. Harris RC, Seifter JL, Brenner BM: Adaptation of Na<sup>+</sup>/H<sup>+</sup> exchange in renal microvillus membrane vesicles: Role of dietary protein and uninephrectomy. J Clin Invest, 74: 1979-1987, 1984.

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