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The Effect of Oral Calcium Load or Verapamil on Gentamicin-Induced Nephrotoxicity

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Summary: Several investigators have reported recently that in rats, oral calcium load is associated with a marked amelioration in gentamicin-induced renal failure. In contrast to these reports, using the same animal model, we could not observe calcium-induced moderation in gentamicin nephrotoxicity as reflected by either urea or creatinine serum concentration or by various renal cortical intracellular enzymatic activities. Similarly, verapamil, a calcium channel blocker, had no effect on the degree of renal failure in these animals. We conclude that manipulation of calcium diet may not be uniformly effective in reducing gentamicin nephrotoxicity. Additional nutritional factors may play a crucial role in achieving the amelioration of this model of toxic nephropathy.

Introduction

Nephrotoxicity due to aminoglycoside treatment is a known clinical complication (1). *Lietman* et al. (2) showed a fall in creatinine clearance in 15% of 214 patients treated with aminoglycosides. Factors that can reduce nephrotoxicity are of great importance as they may enable the use of aminoglycosides with minimal adverse effects (3). In the last decade several investigators have demonstrated that dietary calcium supplementation ameliorated gentamicin-induced renal failure (4–6). The protective mechanism was attributed, at least in part, to a calcium-mediated competitive inhibition of gentamicin binding and uptake along the renal proximal tubular brush border membrane (4). Another aspect of calcium metabolism in uraemia, namely nephrocalcinosis, was recently investigated by *Goligorsky* et al. (7), who found that verapamil prevents renal accumulation of calcium in uraemic rats.

The aim of the present study was:

a) to investigate the effect of calcium loading on the severity of gentamicin-induced renal failure and its impact on various intracellular renal cortical enzyme activities,

b) to evaluate the effects of verapamil on this model of toxic nephropathy.

Materials and Methods

Male Sprague Dawley rats 6–8 week old, weighing 150–200 g were used for all studies. Animals were divided into six groups (tab. 1).

Each group consisted of at least 12 animals. After 10 days all rats were sacrificed. Blood for the various examinations was drawn from the aorta. The serum was analysed for creatinine, calcium, phosphorus and magnesium concentration. The kidneys were removed and processed for biochemical analysis. The renal cortex was placed in a 0.02 mol/l Tris HCl, pH 7.4 buffer (1 : 10 weight to volume ratio) and homogenized in a *Potter-Elvehjem* Glass Teflon homogenizer at 0–4 °C. The homogenate was centrifuged in a Sorvall RC 2B centrifuge at 0–4 °C and the various cellular constituents were obtained by using different centrifugal speeds according to a procedure previously described (8).

Enzyme assays

Aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), *L*-iditol dehydrogenase (EC 1.1.1.14) and orthophosphoric-monoester phosphohydrolase (alkaline optimum) (EC 3.1.3.1) of the homogenate and serum were measured spectrophotometrically with a Gilford Autoanalyser at 30 °C by methods previously described (8, 9). Details of reaction and performance characteristics (within run and run to run) are given (10). Protein content was determined according to *Lowry* et al. (11) Urea was determined enzymatically, according to *Talke & Schubert* (12) and creatinine according to *Fabiny & Ertingshausen* (13). The gentamicin bioassay described by *Bennet* et al. (14) was performed with Muller Hinton Agar plates at pH 8.0, using *Bacillus subtilis* strain AATC 6633; the analytical range was 0.05–3 mg/l with CV < 2.0% at 1.5 mg/l.

Statistical analysis was performed using *Student*'s t test and also by analysis of variance. The results are given in mean ± S.E.M.

Results

Mean urea and creatinine concentrations were similar in the control (Group 1), high calcium diet (Group 3) or verapamil treated (Group 5) rats. The administration of gentamicin resulted in severe renal failure as manifested by the significant increase in serum urea and creatinine concentrations. Neither oral calcium load nor verapamil had any significant attenuating effect on the degree of renal failure (tab. 2).

Mean activities of sorbitol dehydrogenase, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in renal tissue were similar in groups 1, 3 and 5. Treatment with gentamicin was associated with a significant reduction in the activities of these enzymes. The addition of calcium or verapamil did not result in any appreciable attenuation in gentamicin-induced nephrotoxicity (tab. 3).

The relationship between serum urea and creatinine (both reliable markers of glomerular filtration rate) and cellular enzyme activities was investigated. Both serum urea and creatinine showed highly significant inverse correlations with the activities of cellular aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase and lactate dehydrogenase (tab. 4).

No significant decrease was observed in renal tissue gentamicin concentration in rats treated with either oral calcium load or verapamil (tab. 5).

Tab. 1. Experimental design.

No.	Group name	Diet	Subcutaneous injection treatment
1	C = control	Standard rat chow	Twice daily saline 0.2 ml at 08.00 h and 16.00 h.
2	G = Gentamicin	Standard rat chow	Gentamicin 100 mg/kg body weight at 08.00 h and 0.2 ml saline at 16.00 h
3	Ca = Calcium	Standard rat chow* enriched by $CaCO_3$ 40 g/l, Ca-gluconate 5 g/l in drinking water.	Twice daily saline 0.2 ml at 08.00 h and 16.00 h.
4	G + Ca = Gentamicin + Calcium	Standard rat chow* enriched by $CaCO_3$ 40 g/l, Ca-gluconate 5 g/l in drinking water.	Gentamicin 100 mg/kg body weight at 08.00 h and 0.2 ml saline at 16.00 h.
5	V = Verapamil	Standard rat chow	Verapamil 2.5 mg/kg body weight at 08.00 h and 16.00 h.
6	G + V = Gentamicin + Verapamil	Standard rat chow	Verapamil 2.5 mg/kg body weight at 08.00 h and 16.00 h and gentamicin 100 mg/kg body weight at 08.00 h.

* Calcium enrichment started 24 hours before gentamicin treatment.

Tab. 2. Urea (mmol/l) and creatinine (μmol/l) concentration in the various groups (x ± SEM).

	Gr 1 (C) n = 12	Gr 2 (G) n = 14	Gr 3 (Ca) n = 12	Gr 4 (G + Ca) n = 14	Gr 5 (V) n = 12	Gr 6 (G + V) n = 14
Urea	7.1 ± 0.4	38.6 ± 3.9*	6.8 ± 0.3	33.5 ± 4.5*	6.6 ± 0.3	37.3 ± 3.0*
Creatinine	74 ± 10	402 ± 56*	67 ± 56	310 ± 56*	68 ± 7	349 ± 50*

* Significance P < 0.001 for gentamicin addition.

Tab. 3. Renal cortex cellular enzyme values in the various groups (International Units/g protein).

	Gr 1 (C) n = 12	Gr 2 (G) n = 14	Gr 3 (Ca) n = 12	Gr 4 (G + Ca) n = 14	Gr 5 (V) n = 12	Gr 6 (G + V) n = 14
Alkaline phosphatase	174 ± 16	53 ± 6*	183 ± 15	65 ± 8*	171 ± 11	58 ± 6*
Alanine aminotransferase	29 ± 1.3	17 ± 1.5*	29 ± 1.2	19 ± 1.2*	30 ± 1.3	17 ± 1.1*
Sorbitol dehydrogenase	121 ± 10	41 ± 6*	129 ± 12	50 ± 8*	135 ± 9	43 ± 6*
Aspartate aminotransferase	469 ± 11	307 ± 24*	480 ± 12	347 ± 21*	480 ± 16	336 ± 15*
Lactate dehydrogenase	1360 ± 50	1070 ± 60*	1510 ± 50	1320 ± 60*	1510 ± 60	1270 ± 30*

* Significance $P < 0.001$, for gentamicin addition.

Tab. 4. Correlation coefficients relating urea and creatinine values in serum to tissue enzyme values.

	Urea	Creatinine
Aspartate aminotransferase	−0.82	−0.75
Alanine aminotransferase	−0.76	−0.66
Sorbitol dehydrogenase	−0.82	−0.74
Alkaline phosphatase	−0.79	−0.73
Lactate dehydrogenase	−0.66	−0.65

Probability < 0.0001 for all these correlations.

Discussion

Nephrotoxicity is a major adverse effect of aminoglycoside antibiotic agents. The renal dysfunction due to gentamicin treatment in humans has been related to several risk factors (2, 3, 15, 16). The precise intracellular targets that are affected by these agents, leading to renal damage are uncertain. Plasma membrane and lysosomal phospholipases, basolateral membrane Na^+-K^+-ATPase and mitochondrial membranes have been proposed (17–20). Recently gentamicin-induced decreases in mitochondrial cytochrome enzyme concentrations were observed, suggesting the possibility that the renal tubular cell protein synthetic mechanisms are disturbed by this agent (21).

The wide use of these antibiotic agents and their major contribution in the treatment of various infectious processes have initiated a search for treatment modalities which may eliminate or ameliorate their nephrotoxic effect. It has been shown that hydroxyl radical scavengers such as dimethylthiourea, dimethyl sulphoxide and sodium benzoate or deferoxamine (a potent iron chelator) may moderate the gentamicin-induced renal failure in rats (22).

Several investigators have demonstrated that the administration of calcium to rats also ameliorated gentamicin nephrotoxicity (4–6). The mechanism by which this phenomenon is achieved is unclear. It has been suggested that calcium may act as a competitive inhibitor, modifying gentamicin interaction with critical subcellular membrane sites of the renal proximal tubule (6).

In the present study the administration of gentamicin to rats was associated with severe nephrotoxicity as evidenced by impairment of both kidney function (increased urea and creatinine) and metabolism (decrease in various cellular enzymatic activities). In agreement with others (4–6) a high calcium diet was not associated with a lower gentamicin concentration in the renal cortex. However, in contrast to previous reports (2–4), we did not observe any significant amelioration in the degree of nephrotoxicity. This difference in observations can not be explained on a basis of different protocols. Calcium concentration in the diet was similar to that used by others (4–6). The time elapsing between the initiation of calcium loading and the administration of gentamicin, and the dosage of gentamicin were identical to those of a previously used study protocol (6).

Tab. 5. Gentamicin values (mg/g protein) in renal tissue.

	Gr 1 (C) n = 12	Gr 2 (G) n = 14	Gr 3 (Ca) n = 12	Gr 4 (G + Ca) n = 14	Gr 5 (V) n = 12	Gr 6 (G + V) n = 14
Gentamicin	0	39.8 ± 3.9	0	35.8 ± 3.1	0	39.1 ± 3.6

It has been shown that calcium loading in the presence of high sodium intake-induced volume expansion, may not be protective against renal cell injury caused by gentamicin (6). Dietary restriction of protein intake may also moderate to some degree aminoglycoside nephrotoxicity (23). We did not monitor the sodium or protein intake. It may well be that the differing results in our study at least in part, reflect nutritional factors.

Nephrocalcinosis is a common complication of the uraemic state and may play a decisive role in its progression. Gentamicin nephrotoxicity is associated with a significant increase in cellular calcium concentration (6, 24). The main site of its accumulation seems to be within the mitochondria (24). Recently it has been shown that verapamil, a potent calcium-channel blocker may prevent nephrocalcinosis (7). In our study we could not demonstrate any amelioration of gentamicin-induced nephrotoxicity by either kidney function indices or enzymatic-metabolic ones. The administration of verapamil also had no effect on renal tissue gentamicin concentration. Of special interest are the significant correlations between renal function indices such as urea and creatinine, and various enzyme activities. These inverse relationships, which to the best of our knowledge have never been reported previously, demonstrate the direct dependence of glomerular filtration on enzymatic metabolic activities.

We conclude that oral calcium supplementation does not ameliorate gentamicin-induced nephrotoxicity, unless other metabolic or nutritional factors are strictly controlled. Calcium channel blockers do not prevent or moderate the gentamicin-induced renal failure. Changes in metabolism, as reflected by a marked reduction in intracellular enzyme activities, correlate significantly with the degree of gentamicin-induced impairment in glomerular filtration.

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