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Pyridoxal Phosphate a Possible Intervention to Prevent Aminoglycoside Induced Electrolyte Imbalance

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Abstract

Aminoglycosides are very effective against most Gram negative infections, but nephrotoxicity and electrolyte imbalance caused by these compounds pose problems in patients where electrolyte balance needs to be watched closely. The electrolyte imbalance contributes to derangement of the renal functions. This issue has been addressed by changing the dosing schedule and supplementing the patients with these electrolytes. However electrolyte administration and adjusting aminoglycoside doses have their own demerits. In our present study we investigated the effects of Pyridoxal phosphate on electrolyte balance when given in combination with aminoglycosides. Our findings suggest that Pyridoxal phosphate prevents aminoglycoside induced electrolyte imbalance, a finding which has not been reported previously. These results should be considered as an important input regarding prevention of aminoglycoside induced decrease in electrolyte levels. Further studies will help healthcare providers to manage patients more efficiently, in whom electrolytes balance may need to be observed.

Introduction

Recently many of the Gram-negative infections have resurfaced which revived the interest of physicians in the use of aminoglycosides. However this has brought back to light the issues related to these drugs *i.e.* the spectrum of antimicrobial susceptibility and their toxicity [1]. Antimicrobial effect of aminoglycosides is concentration dependent and increases with increasing concentrations of the antibiotic [2]. The killing effect of the aminoglycosides persist even after the medicine has been discontinued [3]. Dose dependent bactericidal effect and post-antibiotic effect make aminoglycosides a good candidate for once-daily dosing in patients with normal renal function and since the dosing intervals are longer, toxicity may also be reduced [4,5]. Nephrotoxicity is a common side effect of the aminoglycosides [6]. Albeit once-daily regimen has improved the state of affairs over what prevailed thirty years ago, the safety of aminoglycosides remains a controversial issue as far as renal tissue is concerned [7,8].

Among other aberrations aminoglycosides decrease the serum levels of various electrolytes like calcium, potassium and magnesium, a phenomenon which is attributed to tubular injury caused by these drugs [9]. Hypocalcaemia has been reported in the patients who were treated with Aminoglycosides [10-12]. To prevent hypocalcaemia, calcium may be added in diet which may reduce the nephrotoxic potential of the drug, but it does not reduce drug accumulation in the renal cortex [13]. Renal potassium depletion has been ascribed to depression of Na^+- K^+-ATPase and decreased serum potassium levels in turn augment the toxic effects of aminoglycosides [14,15]. Hence potassium may be supplemented to avoid its deficiency, especially in patients at risk of acute renal failure, but a falling glomerular filtration rate in the presence of potassium load could result in potentially serious hyperkalemia [16].

Pyridoxal phosphate has nephroprotective effects against various aminoglycosides [17,18]. The electrolyte imbalance contributes to derangement of the renal functions. This issue has been addressed by changing the dosing schedule and supplementing the patients with these electrolytes. However adjusting the proper dose and span of electrolyte administration do pose problems [13,16]. In our present study we investigated the effects of Pyridoxal phosphate on potassium and calcium serum levels when given in combination with Gentamycin and our findings suggest that Pyridoxal phosphate prevents Gentamycin induced electrolyte imbalance.

Material and Methods

Animals

Eight to ten months old male rabbits weighing 1000 to 1500 grams were purchased locally and kept in animal house of the institution after IACUC approval. The animals were fed standard rabbit diet and water was provided *ad libitum*. Twelve hours light and dark cycle was maintained. Fifty four animals were divided in to three groups.

Drugs

Gentamicin sulphate and Pyridoxal-5-phosphate were purchased from Sigma-Aldrich. Fresh solution of each compound was prepared daily. The solution was stored in refrigerator for evening dosage.

Experimental design

Two groups of 18 rabbits each were administered Gentamycin and Gentamycin plus Pyridoxal phosphate intramuscularly whereas the third group was given intra-peritoneal injections of Pyridoxal phosphate alone. All the agents were given at twelve hour-interval (8 a.m. and 8 p.m.) for a period of fifteen days according to following dosage regimen:

- **Group P** was administered with Pyridoxal phosphate in a daily dose of 200 mg/kg.

- **Group G** received Gentamycin in dose of 40 mg/kg/day.
- **Group GP** was administered with Gentamycin and Pyridoxal phosphate simultaneously in daily doses of 40 and 200 mg/kg respectively.

Collection of samples

Blood was collected from the marginal vessels of the ear at day 0 and day 16 by applying Xylene for vasodilatation. Having collected about 6ml of blood in a centrifuge tube, the bleeding vessel was pressed with a sterilized cotton swab till stoppage of bleeding. Serum was separated by centrifuging the collected blood at 3000 rpm and stored at -70° C for later use.

Estimation of serum electrolytes

Serum calcium concentrations were estimated with the orthocresolphthalein method with reagents manufactured by Roche Diagnostics as described earlier [19]. Commercially available reagents (Ciba-Corning, Germany) and Standard (MultiCal) were purchased locally to estimate serum potassium concentration using a flame photometer.

Statistical analysis

All the data was analyzed using statistical package for social sciences (SPSS 13®). A descriptive statistics was used to express the data as mean ± SD. The differences among the groups were examined by using Student's t-test. A value of p less than 0.001 was considered significant.

Results

A significant fall in serum potassium concentration was noticed on day 16 in the animals of group G (3.48 ± 0.38 mEq/L) versus group P (5.17 ± 0.48 mEq/L) where Potassium levels almost remained the same as on day 0. The potassium levels remained high (4.78 ± 0.5 mEq/L) in group GP as compared to group G on day 16 ($P \leq 0.001$) (Figure 1).

Serum calcium levels decreased significantly in group G (7.7 ± 0.29 mg/dL) on day 16 of the study as compared to group P (9.75 ± 0.51 mg/dL) ($P \leq 0.001$). Serum calcium levels in group GP (9.55 ± 0.52 mg/dL) remained comparable with group P on day 16 of the experiment (Figure 2).

Our results suggest that Pyridoxal phosphate prevents the decline in serum electrolyte concentrations associated with Aminoglycosides.

Discussion

In our present study we examined Potassium and Calcium concentrations in the rabbit serum after injecting the animals with Gentamycin and Pyridoxal phosphate either independently or in combination. Our work shows that when given in combination, Pyridoxal phosphate prevents reduction in potassium and calcium serum levels associated with Gentamycin.

We selected daily dose of 40 mg per kg for Gentamycin as most of the research workers preferred doses of either 40 mg [20,21] or 60 mg per kg per day [22,23] in their experimental models. The use of similar doses is based on their similar therapeutic dose, implicated in clinical practice.

In the current study significantly lower levels of serum potassium ($P< 0.001$) were observed in Gentamycin treated animals. These results are in agreement with the previous findings [15,24,25]. There
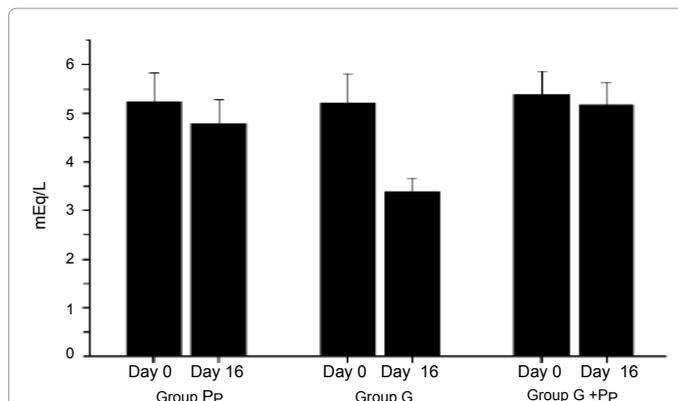

Figure 1: Change in Potassium levels among group P, G and Gentamicin plus Pyridoxal phosphate (GP), during a fifteen day trial in rabbits. Serum Potassium levels decreased by 35% by day sixteen in group receiving only gentamicin while there was only 8% decrease in group receiving gentamicin along with Pyridoxal phosphate on day sixteen. The potassium levels remained high (4.78 ± 0.5 mEq/L) in group GP as compared to group G on day 16 ($P < 0.001$).
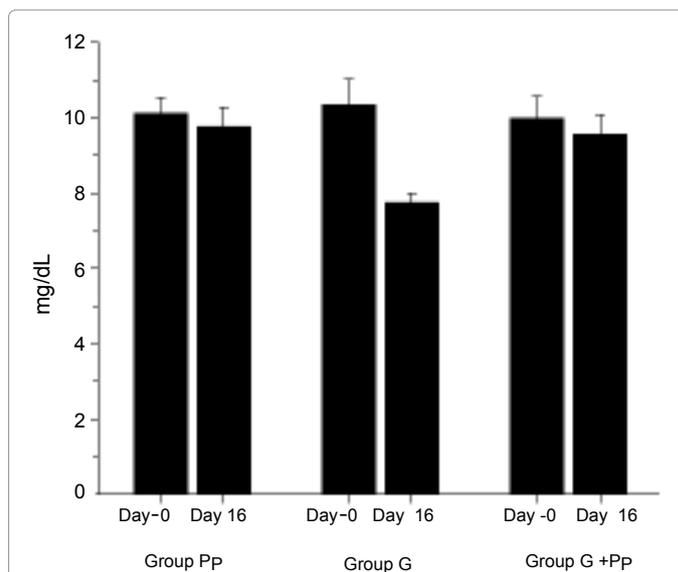

Figure 2: Change in calcium levels in groups P, G and Gentamicin plus Pyridoxal phosphate (GP) during a fifteen day trial in rabbits. Serum calcium levels decreased by 25% by day sixteen in group receiving only gentamicin while there was only 4% decrease serum calcium in group receiving gentamicin along with Pyridoxal phosphate. Serum calcium levels remained high (9.55 ± 0.52 mg/dL) in group GP as compared to group G on day 16 ($P < 0.001$).

was no change seen in the animals which were co-administered with Pyridoxal phosphate, suggesting a protective role of the active form of Vit B$_6$. A significant decrease is also seen in case of calcium when aminoglycosides are administered alone, whereas there is not much change in the serum calcium levels even after fifteen days when Gentamycin was administered along with Pyridoxal phosphate.

Abnormal urinary excretion of sodium, potassium, calcium and magnesium has been described [9]. This may explain the reduction of electrolyte levels when Aminoglycosides are administered alone. However a Schiff base is formed between the amino group of aminoglycosides and the aldehyde group of Pyridoxal phosphate which decreases the nephrotoxicity of the antibiotics. Pyridoxal phosphate prevents the binding of aminoglycosides to the brush boarder

membrane of the proximal tubules and this is due to the interaction of Pyridoxal phosphate and aminoglycosides outside of brush border membrane vesicles [17]. This may prevent the loss of electrolytes and hence maintaining the serum electrolyte levels.

Our study provides an important insight regarding prevention of aminoglycoside induced decrease in electrolyte levels. Further studies will help healthcare providers to decide about managing their patients in which electrolytes balance may need to be watched. We have not studied that how the Pyridoxal phosphate affects the magnesium levels when given along with aminoglycosides. This needs to be carried out in future.

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