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Niacin Ameliorates Hypercalciuria and Hyperphosphaturia Due to Glucocorticoid Administration in Rats

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ABSTRACT

Hypercalciuria and hyperphosphaturia are present in long term and high dose regimens of glucocorticoid therapy. This study aims to evaluate the effect of niacin at its pharmacological dose on calcium and phosphate disturbances due to methylprednisolone administration in growing rats. Twenty one rats were randomly divided into three equal groups and treated as follows for 4 weeks: 1-Normal saline (Control); 2-Methyl Prednisolone (MP) acetate, 3.5 mg kg^{-1} five days a week, SC and 3- MP acetate, 3.5 mg kg^{-1} five days a week, SC + niacin 200 mg kg^{-1} daily by oral gavages. At the end of the experiment, serum and urinary calcium and phosphate assays were performed and calcium content of forth lumbar vertebrate and tibia-fibula bone was determined by atomic absorption method. No significant difference observed in serum calcium or phosphate levels among different groups ($p>0.05$), however an obvious hypercalciuria associated with hyperphosphaturia was present in MP group as compared to control ($p<0.001$). Niacin significantly decreased urinary calcium ($p<0.001$) and phosphate ($p = 0.005$) concentrations as compared to MP group. Calcium level was still significantly higher than control ($p<0.001$), while phosphate decreased even to a lower level than control ($p = 0.005$). Calcium content of forth lumbar vertebrate or tibia-fibula bone of rats remained statistically the same among different groups ($p>0.05$). Niacin at its pharmacological dose can ameliorate hypercalciuria and hyperphosphaturia due to long term and high dose glucocorticoid administration in growing rats without affecting bone calcium content. The possible clinical importance of this effect needs to be clarified in future studies.

Keywords: Glucocorticoid Administration, Niacin, Calcium, Phosphate, Growing Rats, Lumbar Vertebrate, Tibia-Fibula Bone

1. INTRODUCTION

Glucocorticoids (GCs) are widely prescribed to treat immune and inflammatory conditions of different organs including eye, skin, joints, blood, gastrointestinal and respiratory tracts, in veterinary as well as human patients. However, using systemic GCs especially in long term and high dose regimens may be associated with multiple side effects among them are changes in calcium and phosphate homeostasis and bone metabolism. Hypercalciuria is a known adverse effect of treatment with systemic GCs (Bentur *et al*., 2003). Duzen *et al*. (2012) demonstrated that GC treatment induces hypercalciuria just after starting the treatment until the end of it, which promptly improves by the cessation of therapy.

Hypercalciuria is present in 85.7% of people with Cushing's disease; dogs with Cushing's syndrome are 10 times more likely to have calcium-containing uroliths than control dogs (Faggiano *et al*., 2003; Hess *et al*., 1998). Despite hypercalciuria, plasma ionized calcium was normal in people and dogs with hypercortisolism compared with control subjects (Faggiano *et al*., 1982; Ramsey *et al*., 2005). On the other hand, hyperphosphaturia is a common observation following GC therapy or in Cushing's disease (Vrtovsnik *et al*., 1994). Human Cushing's patients have

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hypophosphatemia whereas canine patients have elevated serum phosphorus (Smets *et al*., 2010).

Niacin (Nicotinic acid or vitamin B3), which strongly increases HDL cholesterol levels and has a well-documented anti atherosclerotic effect, has attracted new interest. The discovery of the nicotinic acid receptor GPR109A, which has recently been renamed Hydroxy-Carboxylic Acid (HCA) receptor 2 (HCA_2) (Offermanns *et al*., 2011) has led to new research activities into the mechanisms through which nicotinic acid exerts its pharmacological effects (Gille *et al*., 2008; Kamanna *et al*., 2009a). Recent studies have shown that the nicotinic acid receptor is expressed in various cells including adipocytes, several types of immune cells and keratinocytes. Evidence suggests that nicotinic acid has lipid-independent anti-inflammatory effects (Wu *et al*., 2010; Lukasova *et al*., 2011a). Although it has been demonstrated that niacin lowers serum phosphate in dialysis patients (Muller *et al*., 2007), the effects of this agent on calcium and phosphate imbalance due to GC administration has not been clarified yet.

The present study aims to evaluate the effect of niacin on calcium and phosphate disturbances in growing rats treated with Methyl Prednisolone (MP).

2. MATERIALS AND METHODS

2.1. Animals and Experimental Design

Twenty one female Sprague-Dawley rats with about three weeks of age and a mean body weight of 220 g were purchased from animal house of Shiraz Medical University, Shiraz, Iran. Rats were acclimatized for one week before the beginning of the experiment to the ambient conditions (temperature about 23°C and a 12h/12h, light/dark cycle). Animals had free access to tap water and standard rat chow diet prepared by Razi Vaccine and Serum Research Institute, Shiraz, Iran. After adaptation, rats were randomly divided into three equal groups (n = 7 each) and treated as follows for 4 weeks:

- Normal saline (Control)
- MP acetate (Aburaihan pharmaceutical Co., Tehran, Iran), 3.5 mg kg^{-1} five days a week, SC
- MP acetate, 3.5 mg kg^{-1} five days a week, SC + niacin (Novin Kavosh Mamtir Co., Tehran, Iran) 200 mg kg^{-1} daily by oral gavages

Procedures used in the present study are in accordance with institutional ethical guidelines of School of Veterinary Medicine, Shiraz University, for care and use of laboratory animals in experiments.

2.2. Determination of Calcium and Phosphate Levels in Serum and Urine

At the end of the experiment, rats were fasted over night and voiding urine samples collected in the morning and noon. Blood samples were collected under chloroform anesthesia by cardiac puncture. After centrifugation at 2000 rpm for 20 min, harvested sera were stored in -70°C until use. Calcium and phosphate assays in pooled urinary samples (morning and noon for each animal) and sera were performed by commercial colorimetric kits prepared by Ziest Chem ® Diagnostics, Tehran, Iran.

2.3. Determination of Calcium Content of Forth Lumbar Vertebrate and Tibia-Fibula Bone

After blood collection, animals were euthanized by deepening anesthesia. Forth lumbar vertebrate and right tibia-fibula bone were removed and soft tissues were completely dissected. Bone samples were dried for two weeks at room temperature. Dry-Ashing was performed at 600°C for 8 h and samples were oxidized for 16 h at 100°C bath with a mixture of nitric acid 65% and perchloric acid 70% with 7/3 ratio, there after. Bone calcium content was determined by using an AA670 Shimadzu flame atomic absorption spectrophotometer.

2.4. Statistical Analysis

Data were presented as mean±SD. Data analysis was carried out by using one-way ANOVA and Tukey's multiple comparison tests as the *post hoc* (SPSS 11.5 for windows software). Differences were considered significant at $p<0.05$.

3. RESULTS

3.1. Calcium and Phosphate Levels in Serum and Urine

No significant difference observed in serum calcium or phosphate levels among different groups ($p>0.05$), however; an obvious hypercalciuria associated with hyperphosphaturia was present in MP group as compared to control ($p<0.001$ for both comparisons). Niacin significantly decreased urinary calcium ($p<0.001$) and phosphate ($p = 0.005$) concentrations as compared to MP group. Calcium level was still significantly higher than control ($p<0.001$), while phosphate decreased even to a lower level than control ($p = 0.005$). Data are summarized in **Table 1**.

Table 1. Serum and urinary calcium and phosphate levels of rats in different groups at the end of 4th week of treatment

	Control	MP	MP+N
Serum calcium (mg/dl)	9.26±1.3	10.66±1.23	9.58±0.71
Serum phosphate (mg/dl)	5.52±0.81	6.38±0.79	5.43±0.57
Urinary calcium (mg/dl)	4.04±0.54	11.03±1.13*	7.63±0.74$^{*,\#}$
Urinary phosphate (mg/dl)	62.84±15.9	125±20.17*	29.75±5.42$^{*,\#}$

Control: Normal saline; MP: Methylprednisolone, 3.5 mg kg^{-1} five days a week; MP+N: Methylprednisolone 3.5 mg kg^{-1} five days a week + niacin 200 mg kg^{-1} daily. * and $^{\#}$ signs are used to demonstrate significant difference with control and MP group respectively ($p<0.05$)

Table 2. Calcium content of tibia-fibula bone and forth lumbar vertebrate of rats in different groups at the end of 4th week of treatment

	Control	MP	MP+N
Tibia-fibula bone (mg/cm^3)	297±31	289±12	297±15
Forth lumbar vertebrate (mg/cm^3)	298±44	280±16	307±17

Control: Normal saline; MP: Methylprednisolone, 3.5 mg kg^{-1} five days a week; MP+N: Methylprednisolone 3.5 mg kg^{-1} five days a week + niacin 200 mg kg^{-1} daily. No significant difference observed among groups

3.2. Calcium Content of Forth Lumbar Vertebrate and Tibia-Fibula Bone

No significant difference observed in calcium content of forth lumbar vertebrate or tibia-fibula bone of rats in different groups ($p>0.05$). Data are presented in **Table 2**.

4. DISCUSSION

Prolonged GC use induces osteoporosis; the pathogenesis of this condition is multi factorial and includes GC-induced hypercalciuria (Duzen *et al*., 2012). Kruse *et al*. (1988) observed that GCs induce hyperphosphaturia due to decreased renal phosphate reabsorption not mediated by secondary hyperparathyroidism, as well as marked hypercalciuria in children. These researchers recommended administration of hydrochlorothiazide for correcting hypercalciuria and oral phosphate for hypophosphatemia and replacement of over excreted phosphate from kidneys.

Rats were widely used as a model to study the efficacy of various treatments in the prevention of GC-induced bone loss (Li *et al*., 2007). In growing rats, the prevailing activity on the bone surfaces is modeling, with a linear growth which is rapid until 6 months (Erben, 1966). Our study shows that growing rats clearly exhibit hypercalciuria and hyperphosphaturia due to long term and high dose GC treatment and may be used as a model for evaluation of potential agents with effects on calcium or phosphate balance in this situation. As noted elsewhere, we did not observe significant changes in serum calcium and phosphate levels of MP treated rats. This is consistent with the findings of Wang *et al.* (2002) who observed that administration of MP to rats at the dosages of 2.5, 5, 10 and 20 mg kg^{-1} day^{-1} for 4 weeks does not affect serum calcium and phosphorus concentrations. These reaserches did not evaluate the urinary levels of this ions. It seems that the derangement in calcium and phosphate metabolism due to MP administration is not reflected in their serum concentartion in rats.

Niacin is required at doses of 15-20 mg day^{-1} as a vitamin. However, when it is given in supraphysiological doses, exerts a variety of pharmacological effects (Lukasova *et al*., 2011b). Niacin administration in pharmacological doses seems to be relatively safe. Flushing and gastrointestinal symptoms such as dyspepsia, diarrhea or nausea are the most common unwanted effects of oral niacin therapy. These effects are harmless, never the less, flushing can affect patients' compliance. This effect has been reduced by using extended-release products (Kamanna *et al*., 2009b). Niacin, which strongly increases HDL cholesterol levels and has a well-documented clinical efficacy, has attracted new interest. Few studies are available which have addressed the effect of niacin on phosphorus metabolism. Recently, (Bostom *et al*., 2011) demonstrated that extended-release niacin/laropiprant (a PGD2 receptor antagonist for inhibition of flushing due to niacin) lowers serum phosphorus concentrations in diabetic patients with renal disorders. Moreover, Maccubbin *et al*. (2010) observed a reduction in serum phosphorus concentration of patients who have dyslipidemia and are free of advanced renal disease. As far as we know no study has addressed the effect of niacin on calcium and phosphate disturbances due to GC administration which establishes the rationality for our research.

We observed that niacin significantly reduces urinary calcium and phosphate levels as compared to rats treated with GC alone. The effect of niacin on urinary

phosphate level was so strong where it was reduced to levels even lower than control without any significant change in serum phosphate level as compared to control.

Moreover, GC adiminstration did not result in a significant reduction in calcium content of forth lumbar vertebrate (as a cancellous bone) or tibia-fibula (as a cortical bone) as compared to control group, although a slight reduction was present in both bones. A possible explanation may be the relatively short period of GC treatment, where the loss of calcium from bones for compensation of serum calcium was not still detectable by flame atomic absorption. On the other hand, the effect of niacin on urinary calcium level was not associated with appreciable changes in calcium content of bones as compared to GC group, although this parameter was slightly higher in niacin treated group than GC group specially for lumbar vertebrate. Regardless of the relatively short term of the experiment, this may show that the effect of niacin on amelioration of hypercalciuria due to GC administration may be at least partly due to its plausible effects on other organs which are involved in calcium homeostasis, especially the kidneys. Although this is highly speculative and needs to be further investigated in future studies.

5. CONCLUSION

Niacin at its pharmacological dose can ameliorate hypercalciuria and hyperphosphaturia due to long term and high dose MP administration in growing rats without affecting bone calcium content. The possible mechanisms involved in this effect and its clinical importance needs to be clarified in further studies.

6. ACKNOWLEDGEMENT

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