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Effect of vitamin A supplemented diet on calcium oxalate renal stone formation in rats

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ABSTRACT

Objectives: To study the effect of a vitamin A supplemented diet on calcium-oxalate stone formation in rats and to test its expected action in the dissolution of renal calculi.
Material and methods: Twenty-four male Wistar rats were randomly divided into three groups of eight rats each. The first group (group A) received a normal diet for six weeks. The second group (group B) was fed a lithogenic diet by the addition of ethylene glycol 0.5% to drinking water for three weeks then a normal diet for three weeks. The third group (group C) received the same lithogenic diet for three weeks then a vitamin A supplemented diet 20 times the normal amount (5.1 mg/100 g of diet) at the three last weeks. One day before the end of treatment, each animal was placed for 24 h in metabolic cage in order to collect urine samples and determine the urinary parameters.
Results: The glomerular filtration rate and the urinary excretion of citric acid which fell in group B have been restored in group C.
Conclusions: This study shows that a vitamin A supplemented diet at the rate of 20 times standard ration could improve the renal function by restoring the glomerular filtration rate and by increasing the urinary pH and excretion of citric acid.

Introduction

The impact of nutrition in the renal-stone etiology was underlined by many epidemiological studies (Robertson, 1987; Goldfarb, 1988; Trinchieri et al., 1991). In fact, vitamin A-deficiency in children with low socio-economic status has been associated with stone formation (Kancha and Anasuya, 1992). Moreover, some experiments in animals have shown that a vitamin A deficient diet leads to kidney stone formation (Zile et al., 1972; Grases et al., 1998). Although, to the best of our knowledge, there are no experiments studying the action of a dietary vitamin A supplementation to verify its expected protective effect against kidney stone formation and to elucidate its mechanism of action.

The aim of the present study was to investigate the influence of a vitamin A supplemented diet (20 times the normal amount) on calcium-oxalate renal stone formation induced in rats by a lithogenic diet and to test if vitamin A can dissolve renal calculi already formed.

Material and methods

Animals and diets

Twenty-four male Wistar rats were used in this study. Animals were housed in a temperature (22 ± 2 °C), relative humidity (50%) and photoperiod (12 h light/dark cycle 07:00–19:00) controlled room. Diet and water were given *ad libitum* to rats. After a short period of acclimatization animals were randomly divided into three groups of eight rats each. The first group (group A) received a normal diet for six weeks. The composition of this diet is given in Table 1. The second group (group B) was fed a lithogenic diet by the addition of ethylene glycol 0.5% to drinking water for three weeks then a normal diet for three weeks. Ethylene glycol is known as an important precursor of oxalate and as an effective method for inducing calcium oxalate renal crystals in rats (Moriyama et al., 2009; Tsai et al., 2008). The third group (group C) received the same lithogenic diet for three weeks then a vitamin A supplemented diet 20 times the normal amount (5.1 mg/100 g of diet) at the three last weeks. We have chosen to start vitamin A diet after three weeks in the B group because we would test the expected action of vitamin A in the dissolution of renal calculi which were formed in the three weeks by ethylene glycol. Experiments in animals were ethically acceptable and

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conform to guidelines for animal usage in research. Retinol (purchased from Sigma-Aldrich USA) was used as the source of vitamin A and dissolved in dimethyl sulfoxide.

Sample collection

One day before the end of treatment, each animal was placed in metabolic cage to determine water and food intake and to collect the 24-h urine samples for the ulterior determination of urinary creatinine, urea, calcium, magnesium, uric acid, citric acid and oxalate levels Urine samples were acidified by addition of 50 µl 12 $moll^{-1}$ HCl to dissolve oxalocalcic crystals for calcium and oxalate determination.

Immediately thereafter, rats were weighed and anesthetized. Blood samples were collected in heparinized tubes in order to determine the same biochemical parameters as in urine. Urine and plasma samples were stored at −80 °C until analysis.

Organ analysis

Both kidneys were removed and weighed. The left kidneys were placed for 24 h in an oven at 80 °C for determination of the renal tissue water percentage. The right kidneys were weighed and homogenized in 4 ml of deionized distilled water containing 100 µl of 12 $moll^{-1}$ HCl. After centrifugation, supernatants were collected and stored for oxalate and calcium determinations. The results were expressed per gram of dry tissue, taking into account tissue weight, volume dilution and percentage of water.

Sample analysis

Biochemical determinations of creatinine, oxalate, urea, uric acid, calcium and magnesium were carried out using a KONE 30 automated analyser (Thermoclinical Labsystems, Espoo, Finland). Creatinine was measured by Jaffe method (Randox). glomerular filtration rate (GFR) was estimated by the creatinine clearance method. Oxalate, urea and uric acid were determinated by enzymatic methods using oxalate oxydase (Sigma diag), urease (Eurodiag) and uricase (Biomagreb), respectively. Calcium, magnesium and citric acid levels were mesured by colorimetric methods using Arsenaso III (Eurodiag), Calmagite (Biolabo) and acetic anhydride (Prolabo), respectively.

Statistical analysis

Data are presented as mean ± SD. Fisher's *F*-test and Student's *t*-test were used to test the statistical significance between groups. Differences were considered significant at $p < 0.05$.

Table 1
Composition of standard diet.

Ingredients	Amounts /100 g of diet
Carbohydrates	58 g
Proteins	15 g
Lipids	7 g
Linoleic acid	1 g
Choline	0.5 g
Vitamin A	255 µg
Vitamin D	64 mg
Vitamin C	22 mg
Vitamin B1	290 µg
Vitamin B2	100 µg
Vitamin B6	500 µg
Vitamin B12	0.8 mg
Vitamin PP	7 mg
Vitamin E	3.5 mg
Vitamin K3	100 µg
Calcium	500 mg
Phosphorus	400 mg
Magnesium	120 mg
Iron	15 mg
Copper	500 µg
Zinc	400 µg
Manganese	700 µg
Sodium	0.5 g

Results

The body weight parameters are shown in Table 2. There is no significant difference in the body-weight gain among the three groups. In Table 3 we summarise the 24 h urine biochemical data. Rats in group C showed a significantly higher urine pH in comparison to the other groups.

There was no statistically significant difference in urinary excretion of calcium and oxalate between all groups. Similarly, no significant differences were detected among the three groups in the uric acid and urea urinary excretions and the urine volume (Table 3).

We noticed also that the urinary excretion of citric acid showed a statistically significant decrease in the group B and increase in the group C in comparison to the control group.

The oxalate and calcium contents in renal tissue in different groups are shown in Table 4. In group C we notice a slight decrease especially in the renal tissue accumulation of oxalate in comparison with group B but it is still significantly higher when compared to the rats which were fed a normal diet.

In Fig. 1 we represent the variation of the glomerular filtration rate in the different groups. The GFR which is significantly lower ($p < 0.05$) in group B is not significantly different in group C when compared with group A.

Discussion

Several epidemiological and experimental studies have reported a possible association between vitamin A deficiency and urolithiasis. Vitamin A deficiency in humans was seen to be

Table 2
Rat body weight parameters in control and treated groups.

	Group A (normal)	Group B (EG 3w/N 3w)	Group C (EG 3w/ Vit A 3w)
Initial body weight (g)	128.9 ± 10.6	126.8 ± 10.9	133.6 ± 12.7
Final body weight (g)	238.9 ± 28.7	236.5 ± 18.0	241.5 ± 15.7
Body weight gain (%)	85.3 ± 22.1	85.6 ± 15.0	80.4 ± 13.8

Values are mean ± SD. N: normal diet. EG: ethylene glycol. Vit A: vitamin A supplemented diet. W: weeks.