Effect of Vitamin A Deficiency on Urinary Calculus Formation in Rats

Ravi Kumar Kancha and Adoni Anasuya*

National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania PO, Hyderabad-500007, India

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Summary Effect of vitamin A deficiency on urolithiasis was investigated in male weanling rats. Two groups of rats, namely, experimental and pair-fed controls, were maintained on a basal calculogenic diet high in calcium (9 g/kg diet) for 12 weeks. The diet of the experimental group was devoid of vitamin A. At the end of this period urine was collected for two days and the animals were sacrificed subsequently. Rats in the vitamin A-deficient group exhibited very dense calcium oxalate crystalluria. The incidence and weight of bladder stones were remarkably higher in these rats as well. Besides bladder calculi the experimental group developed renal calculi also. While urinary excretion of calcium, oxalate, and uric acid were increased in the vitamin A-deficient animals, excretion of glycosaminoglycans, citrate, and phosphate were decreased. Inhibitory activity of urine towards the growth of calcium oxalate crystals was markedly reduced in them also. A significant positive correlation between urinary glycosaminoglycans and inhibitory activity of urine and a significant negative correlation between uric acid and inhibitory activity were observed. Subsequent correction of vitamin A deficiency normalized most of the above abnormalities. Involvement of vitamin A deficiency in calculogenesis was clearly demonstrated, and the underlying mechanisms were elucidated in this study.

Key Words: vitamin A deficiency, glycosaminoglycans, inhibitory activity, urinary calculi

Urinary stone formation in man is a multifactorial disorder in which malnutrition plays a significant role. In India a high prevalence of bladder calculi disease is found mostly in boys of low socioeconomic status [1]. Sir Robert...
McCarrison in 1931 suggested the possibility that apart from imbalance of major nutrients, the diets of children prone to urinary calculi formation might also be deficient in vitamin A [2]. A few preliminary studies on experimental animals indicate an association between vitamin A deficiency and formation of calcium carbonate [2], calcium phosphate [3, 4], and calcium oxalate [4] calculi. However, the mechanism by which vitamin A deficiency aggravates calculus formation in rats is not clearly understood.

It is therefore important to delineate the role of vitamin A deficiency in calculogenesis and to elucidate the underlying biochemical mechanism(s). It is also essential to know whether correction of a vitamin A deficiency status helps in reducing the risk of urinary stone formation. To investigate these aspects, we carried out studies on rats fed on a calculogenic diet known to produce calcium oxalate stones [5].

MATERIALS AND METHODS

Thirty-two weanling, male, littermate, Wistar (National Institute of Nutrition, Hyderabad) rats with an average body weight of 42 g were distributed equally into control and vitamin A-deficient groups, by littermate distribution. Both groups of rats received a basal calculogenic diet (Table 1) containing 10% vitamin-free casein (Sigma Chemical Company, St. Louis, MO), 80% sucrose, 5% refined peanut oil (from a commercial source), adequate minerals (E. Merck, India Pvt. Ltd., Bombay) [5] and vitamins (Sigma Chemical Company), except vitamin A [6]. The respective calcium and phosphorus contents were 9.0 and 2.2 g/kg of the diet. Only rats in the control group received a daily dose of 200 IU of vitamin A (Sigma Chemical Company) as retinol palmitate, given orally. Each rat in the control group was pair-fed every day with the same amount of diet consumed by its corresponding littermate in the vitamin A-deficient group.

Table 1. Composition of basal calculogenic diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg of diet</th>
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<tbody>
<tr>
<td>Casein</td>
<td>100</td>
</tr>
<tr>
<td>Sucrose</td>
<td>800</td>
</tr>
<tr>
<td>Refined peanut oil</td>
<td>50</td>
</tr>
<tr>
<td>Salt mixture* [5]</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mixture* [6]</td>
<td>10</td>
</tr>
</tbody>
</table>

*aComposed of the following (g/kg salt mixture): potassium aluminium sulphate, 0.18; calcium carbonate, 560.0; potassium phosphate, 230.0; potassium chloride, 112.0; sodium chloride, 69.0; magnesium carbonate, 35.0; magnesium sulphate, 20.0; ferric phosphate, 21.0; cupric sulfate, 0.9; manganese sulfate, 0.4; potassium iodide, 0.08; sodium fluoride, 0.04.

*bComposed of the following (mg/kg of diet): p-aminobenzoic acid, 110.3; ascorbic acid, 1,017.5; biotin, 0.44; vitamin B12, 0.05; calcium pantothenate, 66.1; choline chloride, 3,715.1; folic acid, 1.98; i-inositol, 110.2; menadione, 49.6; nicotinic acid, 99.2; pyridoxine HCl, 22; riboflavin, 22; thiamine HCL, 22; ergocalciferol, 2,204 IU; vitamin E acetate, 121 IU.

Animals were housed individually in metabolic cages. Distilled deionized water was provided for drinking. Rats were weighed regularly and daily food intake was recorded. The study was conducted in two phases as follows:

**Phase-I.** In the first phase of study the respective diets were fed for 12 weeks. At the end of this period 24-h urine was collected in a polythene vessel containing toluene for two consecutive days, and the volume and pH were recorded immediately. Fresh urine was examined microscopically for crystalluria. The following constituents of urine were estimated: creatinine [7], calcium and magnesium [8], oxalic acid [9], uric acid [10], phosphorus [11], citrate [12], and glycosaminoglycans (GAGS) [13]. Inhibitory activity of urine towards calcium oxalate crystal growth was measured in an *in vitro* system using [1-14C]oxalic acid (BARC, Bombay) [14]. Only analytical grade chemicals were used for these assays. Concentrations of these constituents (except creatinine) were expressed as mg/mg creatinine/24 h.

At the end of the twelfth week, seven rats in each group were sacrificed. All concretions and calculi present in the urinary tract were collected and weighed.

**Liver vitamin A (Dry method).** Liver was collected and processed according to the method of Ames et al. [15]. The vitamin A in the powder was extracted with ether and estimated by the method of Tiews and Zentz [16].

**Phase-II.** The remaining rats in vitamin A-deficient group were supplemented with vitamin A (retinol palmitate, 200 IU/rat/day) for 10 weeks. During this period pair feeding of control rats was continued as described earlier. At the end of the experimental period, 24-h urine was collected for two consecutive days as before and all parameters described in phase-I were again studied. Subsequently all the rats were sacrificed, and incidence and weight of urinary calculi were determined. Then vitamin A content of liver was estimated in all rats.

For statistical evaluation of the data Student’s paired *t*-test was employed.

**RESULTS**

**Growth rate**

By the twelfth week of the experiment, the vitamin A-deficient rats ceased to grow. At the end of this period body weights of vitamin A-deficient rats were significantly low (*p* < 0.001) as compared to pair-fed controls (Table 2). Subsequent supplementation of vitamin A to the deficient rats, however, enhanced their body weight almost to the levels observed in the pair-fed controls.

No significant differences were observed in the weight of either kidney or liver of rats in either group at any stage of the experiment.

**Liver vitamin A content**

Liver A status of the rats was assessed by estimation of the vitamin A content of liver in both groups. Hepatic vitamin A was significantly lower in the experimental group than in the pair-fed controls (*p* < 0.001, Table 2), thus demon-
strating successful induction of vitamin A deficiency in the experimental rats. After supplementation of vitamin A to the experimental group, hepatic content of vitamin A promptly rose close to the levels observed in the pair-fed controls (Table 2).

Incidence and weight of calculi (Table 3)

Incidence of bladder calculi was 29% in the control group and 86% in the vitamin A-deficient one. Besides, 43% of experimental animals developed renal calculi also. The mean weight of bladder calculi in control and experimental groups was 30.0 mg (SE, 2.92) and 151.0 mg (SE, 10.30), respectively (Table 3). Calcium oxalate was the main constituent of these calculi. Figures 1 and 2 show the calculi in an experimental rat.

Incidence of bladder calculi in experimental group after 22 weeks (after rehabilitation) was 22% and the same rats exhibited renal calculi also. Only 11% of the control group developed bladder calculi at this stage.

Properties of urine

Volume. Volume of 24-h urine was similar in control (15.8 ml; SE, 2.00) and experimental (11.7 ml; SE, 1.38) groups.

Table 2. Body, liver, and kidney weights and hepatic vitamin A content of normal and vitamin A-deficient rats before and after rehabilitation.

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>(n=7)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>189.0±5.10</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>5.8±0.25</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.6±0.06</td>
</tr>
<tr>
<td>Vitamin A (µg/g)</td>
<td>192.0±10.30</td>
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</table>

Values are mean±SE. **p<0.001 vs. Phase I control.

Table 3. Percent incidence and weight of urinary stones in control and vitamin A deficient rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vitamin A deficient</th>
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<tbody>
<tr>
<td></td>
<td>Bladder</td>
<td>Kidney</td>
</tr>
<tr>
<td>12 weeks (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Incidence</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>30.0±2.92</td>
<td>0</td>
</tr>
<tr>
<td>22 weeks (n=9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Incidence</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>57.0±0.00</td>
<td>0</td>
</tr>
</tbody>
</table>

Weight of stones: mean±SE. *Vitamin A-deficient rats were rehabilitated for 10 weeks after 12 weeks on the deficient diet.
EFFECT OF VITAMIN A DEFICIENCY

pH. There was no significant difference in the pH of urine between control (7.9; SE, 0.03) and experimental (8.0; SE, 0.03) animals.

Crystalluria. Compared with controls, vitamin A-deficient rats exhibited much denser calcium oxalate crystalluria comprising bigger crystals and their massive aggregates (Figs. 3 and 4).

Urinary constituents (Table 4). Twenty-four hour excretion of urinary creatinine was significantly low ($p<0.01$) in the experimental group as compared with the control value. Concentrations of other urinary constituents expressed in terms of creatinine (mg/mg creatinine/24 h) showed the following changes: Vitamin A deficiency resulted in a significant ($p<0.001$) increase in 24-h excretion of calcium, oxalate, and uric acid. On the other hand, avitaminosis A resulted in decreased excretion of GAGS ($p<0.001$), citrate ($p<0.001$), and phosphorus ($p<0.005$). In vitro inhibitory activity of urine towards calcium oxalate crystal growth (referred to as “Inhibitory activity” in this paper) was significantly lower ($p<0.001$).
0.001) in the vitamin A-deficient rats than in the controls.

A significant ($p<0.05$) positive correlation ($r=+0.98$) between urinary GAGS and inhibitory activity and a significant ($p<0.001$) negative correlation ($r=-0.69$) between urinary uric acid and inhibitory activity were observed in the vitamin A-deficient rats.

Almost all the biochemical abnormalities observed in vitamin A-deficient rats could be reversed promptly by correction of the vitamin A deficiency (Table 5).
EFFECT OF VITAMIN A DEFICIENCY

DISCUSSION

That vitamin A deficiency superimposed on a calculus-producing diet does enhance the risk of calcium oxalate calculi formation was amply demonstrated in this study. Factors responsible for such an effect were also identified.

Vitamin A deficiency resulted in crucial changes in certain properties of urine. Thus the excretion of high risk components of urine, namely, oxalate, calcium, and uric acid, were increased. Simultaneously, a significant reduction in the concentration of GAGS, citrate, and phosphate, all of which are known inhibitors of calcium oxalate crystal growth [14], were also observed in these rats. The net effect of these two major opposing changes was a drastic increase in the risk of stone formation and growth.

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The effect of abnormal excretion of each one of the above components of urine was obvious at discrete stages of urinary stone formation in the vitamin A deficiency state. Thus, due to a high concentration of both oxalate and calcium, urine attained supersaturation with respect to these ions; and, as a result, very dense calcium-oxalate crystalluria (which is an essential prerequisite for subsequent stone formation) was encountered in these vitamin A-deficient animals. Thus formed, calcium oxalate crystals had ample opportunity to grow further and to form aggregates in the urine due to inadequate levels of inhibitors of calcium oxalate crystal growth and aggregation, namely, GAGS, citrate, and phosphate.

Our observations are in agreement with other reports that vitamin A deficiency results in hypercalciuria, hyperoxaluria [4], and reduced urinary phosphate concentration [17].

Of the known compounds present in urine that inhibit the growth of calcium oxalate crystals, GAGS are reported to be the most potent and thus the important in this respect [18]. The inhibitory activity of GAGS is believed to lie in their ability to bind to the active growth site of crystals, thereby blocking crystal growth and aggregation [19].

In the vitamin A-deficient group a significant drop in in vitro inhibitory activity of urine and a positive correlation between concentration of GAGS and inhibitory activity were observed. This is a definite proof of the role of GAGS as the most potent inhibitor of calcium oxalate crystal growth. It is of interest to note that this inhibitory activity was also influenced by the concentration of urinary uric acid as evidenced by significant negative correlation between these two parameters. In addition, enhancement of the uric acid concentration of control rat’s urine to the level observed in vitamin A-deficient rats led to a prompt decrease in original inhibitory activity of control urine (Table 6). But uric acid itself did not affect the level of residual radioactivity in the metastable solution 48 ± 1.55% (solution only) vs. 46.5 ± 1.23 (solution + uric acid). Therefore, the mode of action of uric acid on inhibitory activity appears to be not direct, but mediated through GAGS present in urine. As suggested by Coe et al. [20], uric acid might mask the inhibitory action of GAGS. Thus enhanced excretion of uric acid observed in vitamin A-deficient rats may indirectly aid the formation of calcium oxalate crystals in the urine. As a result of all these changes resulting from vitamin A deficiency, a crucial stage is set for subsequent formation and growth of calculi.

While the consequences of the several changes discussed above in vitamin A deficiency status on urinary calculi formation can thus be visualized, the reasons for such changes are not yet completely clear. The following observations can

Table 6. Effect of addition of uric acid on in vitro inhibitory activity of urine.a

<table>
<thead>
<tr>
<th>Vitamin A deficient</th>
<th>Control</th>
<th>Control + Uric acid</th>
</tr>
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<tbody>
<tr>
<td>42.0 ± 0.91**</td>
<td>64.1 ± 1.17**</td>
<td>54.9 ± 1.32**</td>
</tr>
</tbody>
</table>

Values are mean ± SE. **p < 0.001. n = 6 in each group. aInhibitory activity is expressed as % 14C-oxalate residual radioactivity in a metastable solution of calcium oxalate.
partly explain the fall in the urinary GAGS observed in this study. Reduced excretion of GAGS observed in the vitamin A-deficient animals could be due to reduced synthesis of these compounds. Latif et al. [21] reported reduced urinary excretion of GAGS in vitamin A-deficient children, which might be due to reduced synthesis of GAGS [22]. This aspect is currently being investigated by us.

Our recent observations [23] show that intestinal absorption of both calcium and oxalate was increased in vitamin A-deficient rats. This can partly explain hyperoxaluric and hypercalcuiuric states observed in vitamin A deficiency. Possible changes in biosynthesis of oxalate, renal handling of citrate, urate, and phosphate in vitamin A deficiency are also under investigation.

This study also clearly demonstrated that enhanced calculogenic potency of urine brought about by vitamin A deficiency is reversible. This again is an important observation of practical significance.

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REFERENCES


