Effect of Vitamin A Deficiency on Oxalate Uptake by Rat Intestinal Brush Border Membrane Vesicles (BBMV) and Its Contribution towards Urolithiasis

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Summary The intestinal uptake of $[^{14}C]$oxalate, $[^{14}C]$glyoxylate, and $[^{14}C]$glycolate are studied in brush border membrane vesicles (BBMV) isolated from vitamin A-deficient and pair-fed control rats. The data obtained indicate that oxalate and its precursors are transported across the BBMV by passive diffusion. The intestinal uptake of glyoxylate and glycolate remains unaltered in vitamin A deficiency, while uptake rate of oxalate was significantly increased ($p < 0.01$) in vitamin A-deficient rats as compared to pair-fed controls. In conclusion, the results indicate that vitamin A deficiency leads to hyperabsorption of oxalate through the gut.

Key Words vitamin A deficiency, oxalate uptake, intestinal BBMV, urolithiasis

It is indisputable that hyperoxaluria is conducive to stone formation and has been receiving greater attention as it is one of the most cogent risk factors in the initiation of stone episode (1-5). Oxaluria has invariably been associated with the nutritional imbalances, especially vitamin deficiencies, which are quite common in India and other developing countries. Although vitamin A deficiency has been described as an important etiological factor of urolithiasis (6), its biochemical mechanism has not yet been elucidated. The primary cause of hyperoxaluria and nephrolithiasis is attributed to higher absorption of exogenous oxalate (7-10); previous work from this laboratory has demonstrated an increased rate of oxalate uptake by intestinal mucosal cells using a tissue accumulation method in vitamin A-deficient rats (11), thus indicating that this may be a possible mechanism responsible for such a change at the membrane level. Recently, pure brush border membrane vesicles (BBMV), which could overrule the drawbacks of isolated cells, have been widely used to study transport characteristics of various nutrients (12-14). Furthermore, the transport properties of the brush border membrane (BBM) can be studied without interference or participation of cellular metabolism and
energy or intracellular compartmentation. Therefore, in the present study, intestinal BBMV have been employed to investigate the effect of vitamin A deficiency on intestinal uptake of oxalate.

MATERIALS AND METHODS

Male Wistar rats (40-50 g body weight) were divided into the following groups of 8-10 animals each and were kept on their respective dietary regimens for 5 weeks.

Group I: Fed vitamin A-deficient diet ad libitum.
Group II: Pair-fed with group I + 150 IU of retinyl acetate/rat/day orally in refined groundnut oil.

At the end of the experimental period, when clinical symptoms of vitamin A deficiency—decrease in body weight, keratinization of eyes, etc.—became apparent, the overnight fasted animals were sacrificed under light ether anesthesia. Vitamin A content in the liver was used as an index of vitamin A deficiency (15).

Preparation of intestinal brush border membrane vesicles. Briefly, starting from the ligament of Trietz, a portion (approximately 20 cm) of gut was flushed with ice-cold normal saline and everted using a stainless steel rod. The mucosa was scraped with the help of a glass slide, weighed, and 3% homogenate was prepared in homogenizing buffer (50 mM mannitol, 2 mM Tris-HEPES, pH 7.1) in a Hotline mixer for 2 min at 4°C. Magnesium chloride was added to final concentration of 10 mM. The BBMV were prepared by the differential centrifugal technique (13, 14).

The purity of BBMV was checked by assessing the specific activities of the BBM marker enzymes sucrase (16) and alkaline phosphatase (17). Contamination by basolateral membrane was ascertained by measuring specific activity of Na⁺, K⁺-ATPase (18).

Uptake of glucose, oxalate, glyoxylate, and glycolate. The uptake of various nutrients into intestinal BBMV was measured by millipore filtration technique (12). The uptake buffer consisted of 100 mM D-mannitol, 2 mM Tris-HEPES, 100 mM NaCl, pH 7.4. Uptake assay was initiated by addition of 20 µl of BBMV preparation (80-100 µg protein) to incubation medium containing 1.0 mM potassium oxalate or sodium glycolate or sodium glyoxylate or D-glucose with traces of respective radioactive substrates (0.1 µCi/ml). Incubations maintained at 25°C were terminated at specified times by addition of 50 volumes of ice-cold stop solution containing 100 mM D-mannitol, 100 mM NaCl, 2 mM Tris-HEPES, pH 7.4. Following filtration of suspension through 0.45 µm nitrocellulose filters under low vacuum, the trapped BBMV were again washed with 2.5 ml of ice-cold stop solution, and filters were counted for radioactivity in a Packard Scintillation Counter.

Urine analysis. Twenty-four-hour urine was collected in a metabolic cage and analyzed for oxalate (19) and creatinine (20).

Statistical analysis. Student t-test was used for statistical analysis of the data.
RESULTS

The rats kept on the vitamin A-deficient diet for 35 days showed a significant decrease ($p<0.01$) in their body weight as compared with pair-fed controls (Table 1). The experimental production of vitamin A deficiency was biochemically confirmed by a significant ($p<0.001$) decline in the hepatic vitamin A levels in vitamin A-deficient rats (Table 1). A significant hyperoxaluria ($p<0.01$) was also observed in the hypovitaminosis A group as compared to pair-fed controls. About 20-fold increase in the specific activity of sucrase and 14-fold in the activity of alkaline phosphatase were observed in the BBMV preparation as compared with its respective homogenates, both in pair-fed control and vitamin A-deficient rats. Contamination by the basolateral membranes was ruled out by the assay of Na$^+$, K$^+$-ATPase in the BBMV, which showed 92–95% decrease with respect to its homogenate (Table 2).

Table 1. Effect of vitamin A deficiency (of 35 days duration) on body weight, liver weight, hepatic vitamin A content, and urinary oxalate excretion in rats.

|                          | Pair-fed control | Vitamin A-deficient |
|--------------------------|------------------|---------------------|
| Initial body weight (g)  | 55.00±2.83       | 49.50±0.46          |
| Final body weight (g)    | 143.25±5.93      | 114.25±3.00**       |
| Liver weight (g/g organ weight) | 3.49±0.11       | 3.76±0.10           |
| Hepatic vitamin A (IU/g organ weight) | 144.43±4.03   | 2.76±0.03***       |
| Urinary oxalate (mg/mg creatinine) | 0.306±0.02   | 0.648±0.06**       |

Values are $M±SE$ of 8–10 animals. **$p<0.01$, ***$p<0.001$ as compared to pair-fed controls. 1 IU defined as equivalent to 0.344 $\mu$g of pure vitamin A acetate.

Table 2. Biochemical assessment of the purity of brush border membrane vesicles preparation from the intestine of pair-fed control and vitamin A-deficient rats.

| Enzyme                  | Homogenate | Brush border membrane vesicles | Enrichment factor$^a$ |
|-------------------------|------------|--------------------------------|-----------------------|
|                         | Pair-fed control | Vitamin A-deficient | Pair-fed control | Vitamin A-deficient | Pair-fed control | Vitamin A-deficient |
| Sucreose (3)            | 0.045±0.010  | 0.049±0.007                  | 0.800±0.060          | 0.867±0.023         | 18.73            | 17.69              |
| Alkaline phosphatase (3) | 0.073±0.011  | 0.040±0.013                  | 1.140±0.072          | 0.728±0.041         | 15.61            | 14.46              |
| Na$^+$,K$^+$-ATPase (3)  | 0.360±0.050  | 0.390±0.020                  | 0.044±0.007          | 0.044±0.007         | 0.11             | 0.10               |

Values are $M±SD$ of 8–10 observations. Number of experiments is given in parentheses. $^a$The enrichment factor is the ratio of specific activities in the final brush border membrane and the homogenate.

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Effect of vitamin A deficiency on Na\(^+\)-dependent D-glucose uptake by rat intestinal (jejunum) BBMV.

The viability of the intestinal BBMV was routinely assessed by studying Na\(^+\)-dependent D-glucose uptake. In the presence of NaCl gradient across the membrane (out\(>\)in), a transient accumulation of D-glucose against its own concentration was observed. The results obtained thus confirm (Fig. 1) the purity and biochemical viability of BBMV.

The kinetics of oxalate uptake were studied by measuring oxalate influx as a function of varied extravesicular oxalate concentration (0.1–1.0 mM). The rats from both pair-fed control and vitamin A-deficient group exhibited a linear positive relationship between oxalate-uptake rate and oxalate concentration (Fig. 2), thus suggesting that oxalate is transported through intestinal BBM passively. However, the rate of oxalate uptake was significantly high in vitamin A-deficient rats as compared to pair-fed controls as indicated by their slope lines (control, 0.63±0.04; vitamin A deficient, 0.87±0.03), which suggested that vitamin A deficiency leads to hyperabsorption of oxalate through the gut.

The intestinal uptake of two important metabolic precursors—glycolate and glyoxylate—was also studied in both pair-fed control and vitamin A-deficient groups. The glyoxylate uptake was found to be a linear function of substrate concentration in the incubation medium with no evidence of saturation over the concentration range studied (0.1–1.0 mM). Also, vitamin A deficiency did not produce any significant effect on the uptake rate of glyoxylate as determined from the slope values (control, 0.39±0.05; vitamin A deficient, 0.42±0.03). Similar to glyoxylate and oxalate, glycolate was also found to be transported through intestinal epithelial membrane by passive diffusion in both the groups. Glycolate uptake in vitamin A-deficient rats (5.04±0.51 nmol/mg protein) was not significantly different from controls (4.89±0.54 nmol/mg protein). Also, no difference was observed between slope values of the two groups (control, 0.46±0.01; vitamin

**Fig. 1.** Effect of vitamin A deficiency on Na\(^+\)-dependent D-glucose uptake by rat intestinal (jejenum) BBMV.
DISCUSSION

The oxalate-, glycolate-, and glyoxylate-uptake studies were performed with BBMV isolated from rat jejunum, as this tissue exhibited maximum absorption of oxalate (21). The mean increase in the specific activity of a typical BBM enzyme, namely sucrase (Table 2), is in accordance with the 21-22-fold purification reported by several workers (13,14). The viability of intestinal BBMV as assessed from Na⁺-dependent D-glucose uptake (Fig. 1) is in excellent agreement with the cotransport mechanism proposed by others (22,23) for this system. Our observations that oxalate is transported across the microvillar membrane by passive diffusion are parallel with earlier observations from this laboratory (9, 24, 25). A passive and non-energy-dependent transport of oxalate across rabbit intestine has also been described earlier (26). Since oxalate is a divalent anion, its translocation across the lipid bilayer is very slow in the absence of specific transport carriers. Therefore, it might be assumed that organic anions are crossing the intestinal boundary by way of aqueous channels or passive diffusion. Various organic acids—hippuric acid, p-aminohippuric acid, and sulfonilic acid—are also known to be passively diffused in the rat intestine (27). Intestinal absorption of oxalate has been shown to follow a passive diffusion in the BBMV (10). The negative membrane potential imparted to the microvillus surface by oxalate could explain the stimu-
lation of oxalate transport in vitamin A-deficient rats. The increased absorption of oxalate from intestine in vitamin A deficiency may partly be due to increased permeability of oxalate through enterocyte membrane as a consequence of change in chemical composition of BBM as reported earlier (24). Earlier work from our laboratory reported a 20 to 40% increase in oxalate influx in vitamin B<sub>6</sub>-deficient rats as compared to pair-fed controls, while thiamine deficiency of 4 and 6 weeks duration did not produce any significant changes in the passive influx of oxalate (9), suggesting that the mode of action of vitamin A is quite different from other vitamins. The low content of the dry matter and the physical condition of the small intestine in vitamin A-deficient animals appeared to be a reflection of change in the thickness of the intestine wall, which may effect the in vitro transfer of various nutrients by the intestine (28).

The glyoxylate and glycolate are the major precursors of oxalate and are readily absorbed through the intestine. The glycolate-induced hyperoxaluria in laboratory animals has been widely used as a model for hyperoxaluria and stone formation. Our results suggest a passive diffusion mechanism for intestinal uptake of [<sup>14</sup>C]glycolate and [<sup>14</sup>C]glyoxylate, which corroborates earlier reports (9, 29). The difference in chemical structure of glyoxylate or glycolate as compared to oxalate may explain its unaltered translocation through intestine in vitamin A deficiency. Similar results were obtained (9) where pyridoxine or thiamine deficiency failed to produce any alteration in absorption pattern of these precursors of oxalate. These observations thus exclude the implication of dietary glycolate or glyoxylate as major contributing factors to hyperoxaluria in vitamin A deficiency.

The retinyl acetate supplementation to vitamin A-deficient rats could reverse the changes in the intestinal uptake of oxalate. These results lend further support to the earlier claims that vitamin A is specifically required in the differentiation of intestinal epithelial cells which are the main target, for admittance of various nutrients to the body (30, 31). The present work thus suggests that vitamin A deficiency, which stimulates the absorption of exogenous oxalate through the gut, must be seen as an aggravating factor that increases the prevalence of stone formation in a general population afflicted with a marginal deficiency of this vitamin.

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