Urol Res (1991) 19:305–308

Urological Research

Effects of dietary calcium, magnesium and phosphorus on the formation of struvite stones in the urinary tract of rats

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Accepted: January 1, 1991

Summary. After feeding various diets we studied the effects of dietary calcium, magnesium and phosphorus on the formation of struvite stones in rats with urinary tract infections, and also studied the effects of the administration of vitamin D_3 and aluminium gel on stone formation. A low-magnesium diet decreased urinary magnesium and prevented stone formation, but a medium-calcium diet did not significantly decrease stone weight. A high-calcium diet decreased urinary phosphorus and inhibited stone formation. A high-calcium and high-phosphorus diet decreased urinary excretion of magnesium and inhibited stone formation. Although the administration of vitamin D_3 did not inhibit stone formation, aluminium gel decreased the urinary level of phosphorus and prevented stone formation. A marked decrease in urinary magnesium and/or phosphorus may prevent struvite stone formation in rats with urinary tract infections.

Key words: Diet – Calcium – Magnesium – Phosphorus – Struvite stones – Rat

Stones caused by urinary tract infections in humans are composed mainly of a mixture of struvite and carbonate apatite [10, 16], and the ratio of the two components depends on the urinary concentration of calcium, magnesium and phosphate. On the other hand, experimentally induced infection stones in rats are composed of almost pure struvite [6, 12, 14, 15, 17]. The difference in composition of infection stones between humans and rats is thought to result from urinary constituents or diet. Humans excrete more calcium than magnesium in the urine, while rats excrete more magnesium than calcium [5].

The effects of diet on calcium stone formation have been studied experimentally [2, 4] as well as clinically [1, 9, 11], but the only reports available on the effects of diet on experimentally induced struvite stone formation in rats concern non-infectious stones [5, 18].

This study examined the effects of various diets on stuvite stone formation in rats with urinary tract infections and the effects of the administration of vitamin D_3 and aluminium gel on stone formation.

Materials and methods

Bacteria

Proteus mirabilis was isolated from the urine of a patient with a urinary tract infection.

Animals

Male Wistar strain rats (specific pathogen free, 7 weeks old, weighing 180–200 g) were used in all experiments.

Diets and drugs

Five special types of diet were prepared as shown in Table 1: a standard maintenance diet (MF), a medium-calcium diet (F-1), a low-magnesium diet (F-2), a medium-calcium/low-magnesium diet (F-3), a high-calcium diet (F-4) and a high-calcium/high-phosphorus diet (F-5).

Vitamin D_3 (0.5 μg) was administered orally to rats by a gastric tube every other day. An aluminium diet was prepared containing 4.3% aluminium gel in MF.

Induction of stone formation in rats

Urinary tract infections were induced in rats by the method of Vermeulen and Goetz [17]: zinc discs, dipped into a saline suspension containing 10^8 *P. mirabilis* per ml, were implanted into the bladder.

Protocol for studies on the effects of diet on stone formation

For 3 days prior to implantation of septic discs, rats were housed in metabolic cages and maintained on water and special diets ad libitum, and 1 day before and 6 days after implantation, urine specimens were collected for determination of urinary calcium,

Table 1. Dietary content and urinary excretion of calcium, magnesium and phosphorus in uninfected rats

Nutrient content (g/100 g)					Urine (mg/day)		
Diet	Ca	Mg	P	Ca/Mg	Ca	Mg	P
MF	1.1	0.25	0.83	4.4	1.3 ± 0.6[a] ($n = 8$)[b]	5.3 ± 2.0	13.1 ± 5.7
F-1	2.2	0.25	0.83	8.8	2.1 ± 0.6* ($n = 5$)	3.3 ± 1.6	36.3 ± 8.3**
F-2	1.1	0.06	0.83	18.3	0.9 ± 0.5 ($n = 5$)	1.2 ± 0.7**	67.7 ± 27.1*
F-3	2.2	0.06	0.83	36.7	1.6 ± 1.1 ($n = 4$)	0.6 ± 0.5**	14.0 ± 2.8
F-4	11.0	0.25	0.83	44.0	6.6 ± 3.4* ($n = 5$)	5.8 ± 2.1	2.7 ± 1.1**
F-5	11.0	0.25	8.30	44.0	1.8 ± 1.6* ($n = 10$)	0.8 ± 0.8*	88.5 ± 30.0**

[a] Values are expressed as means ± SD
[b] The number in parenthesis represents the number of specimens analyzed
* $P < 0.05$; ** $P < 0.01$ (significance of difference from MF group)

Table 2. Urinary calcium, magnesium and phosphorus in rats with urinary tract infections

Diet	Number of rats	Vol (ml)	Urinary excretion (mg/day)		
			Ca	Mg	P
MF	14	27 ± 10.0[a]	0.5 ± 0.4	1.0 ± 0.8	12.8 ± 7.9
F-1	10	21.8 ± 9.7	1.5 ± 1.2*	1.0 ± 1.0	26.2 ± 14.3**
F-2	10	15.8 ± 2.8**	1.1 ± 0.8*	0.6 ± 0.5	65.5 ± 11.7**
F-3	11	15.6 ± 6.0**	0.8 ± 0.9*	0.5 ± 0.5	4.9 ± 2.1**
F-4	10	8.7 ± 5.4**	5.9 ± 6.0**	2.6 ± 1.7	1.9 ± 1.1**
F-5	10	23.4 ± 6.1**	0.9 ± 0.4	0.1 ± 0.1**	91.7 ± 24.2**
MF + D_3	5	32.6 ± 6.8	3.0 ± 0.7**	2.3 ± 1.7	18.3 ± 5.9
MF + Al	6	16.5 ± 7.3*	1.3 ± 0.6**	3.4 ± 2.3	2.5 ± 2.1**

[a] Values are expressed as means ± SD
* $P < 0.05$; ** $P < 0.01$ (significance of difference from MF group)

magnesium and phosphorus levels. Rats were sacrificed on the 7th day after implantation and blood specimens were collected for determination of serum calcium, magnesium, phosphorus and blood urea nitrogen (BUN). The urine aspirated from the bladder was inoculated on MacConkey and CLED plates (Dip slide methods, Uricult, Daiichi Chemical Ind., Tokyo, Japan), and the approximate number of bacteria was determined. Urine pHs were determined using test papers (Toyo-Roshi, Tokyo, Japan). Bladder stones were dried overnight at 37°C in vacuo and were weighed and analyzed by an infrared spectrometer. The gross appearance of the kidneys was observed macroscopically.

Urinary and serum calcium, magnesium and phosphorus, and BUN were measured using a commercial kit-Calcium C-Test, Magnesium B-Test, P-Test and Urea N-Test (Wako Pure Chemical Ind., Osaka, Japan).

Rats which died before the completion of the study or failed to be infected were excluded. Student's *t*-test was used for statistical analysis of data, and *P* values lower than 0.05 were considered significant.

Results

Effects of special diets on urinary calcium, magnesium and phosphorus in uninfected rats

As shown in Table 1, the uninfected rats fed MF excreted more magnesium than calcium. In the F-1 diet group the urinary excretion of calcium and phosphorus increased significantly compared with that in the control MF group. In the F-2 diet group urinary magnesium decreased but urinary phosphorus increased. In the F-3 diet group urinary magnesium excretion was markedly decreased and the urinary calcium/magnesium ratio increased to greater than 1.0. In the rats fed the F-4 diet, urinary phosphorus markedly decreased while urinary calcium increased significantly. In the F-5 group urinary calcium was unchanged, urinary magnesium decreased significantly, and urinary phosphorus greatly increased.

Effects of special diets, aluminium gel or vitamin D_3 on struvite stone formation

Two rats in the control group, one in the MF + D_3 group, and one in the MF + Al group died before the completion of the study. Urine cultures of all rats tested revealed more than 10^5 *P. mirabilis* per ml. Urine pH of the control group was 7.46 ± 0.36 (Mean ± SD). No differences in urinary pH between the control and each experimental group were noted.

Table 2 shows the urinary excretion of calcium, magnesium and phosphorus in the infected rats fed various diets, or administered vitamin D_3 or aluminium gel. Urine volume increased more in infected rats than in

Table 3. Serum levels of calcium, magnesium and phosphorus, BUN and weights of stones rasts with urinary tract infections

Diet	Number of rats	Serum (mg/dl)				Stone weight (mg)	Incidence of pyelonephritis or renal abscess
		Ca	Mg	P	BUN		
MF	16	9.5 ± 1.5[a]	3.4 ± 0.8	10.1 ± 2.1	30.6 ± 21.1	50 ± 27	14/16
F-1	10	10.5 ± 0.6 (5)[b]	2.0 ± 0.3** (5)	7.3 ± 0.7** (5)	22.6 ± 6.4	35 ± 12	7/10
F-2	10	10.4 ± 0.5* (5)	1.5 ± 0.4** (6)	6.7 ± 0.5** (6)	18.4 ± 4.3*	1 ± 2**	5/10
F-3	11	9.5 ± 1.7 (5)	2.2 ± 0.5** (5)	9.1 ± 1.0 (5)	–	2 ± 3**	3/11
F-4	10	12.2 ± 0.6**	3.7 ± 0.7	4.9 ± 1.0**	44.3 ± 40.1**	5 ± 7**	5/10
F-5	10	8.7 ± 1.1	3.4 ± 0.3	12.6 ± 1.7**	45.4 ± 15.7*	1 ± 2**	Calcinosis 3/10
MF + D_3	7	11.6 ± 0.5	3.7 ± 0.7	8.5 ± 1.3	22.9 ± 10.0	57 ± 30 (5)	–
MF + Al	6	10.2 ± 0.5	2.7 ± 0.3	8.0 ± 2.4	30.5 ± 10.5	3 ± 6**	3/6

[a] Values are expressed as means ±SD
[b] The number in parenthesis represents the number of specimens analyzed
* $P < 0.05$; ** $P < 0.01$ (significance of difference from MF group)

uninfected rats, but the urinary excretion of calcium and magnesium decreased somewhat in infected MF rats. When compared with the infected MF group, urinary calcium and phosphorus levels increased in F-1 and F-2 rats, a result which is similar to the response obtained in uninfected F-1 and F-2 groups. Urinary phosphorus decreased in infected F-3 rats, differing from the unresponsive uninfected case. Urinary excretion of calcium, magnesium and phosphorus in infected F-4 or F-5 rats was similar to that in the corresponding uninfected F-4 or F-5 rats. Rats that received vitamin D_3 had significantly increased urinary calcium and urinary magnesium tended to increase. Urinary excretion of calcium increased and phosphorus excretion decreased in the rats that received aluminium gel.

Stone weight, serum data and histological findings are summarized in Table 3. The mean stone weight in the F-2, F-3, F-4, F-5 and MF + Al groups was significantly less than in the control group. These small stones were made up of calcium carbonate and not struvite. The struvite stones formed in F-1 rats were marginally smaller than the stones formed in MF rats. The bladder stones that formed in rats receiving vitamin D_3 were similar in size to those observed in the control rats.

The changes in serum levels of calcium, magnesium and phosphorus in each group were determined. In the F-1 and F-2 groups, both serum magnesium and phosphorus were significantly lowered. In the F-3 group serum magnesium was lowered. In the F-4 group, serum calcium was markedly increased and phsophorus was decreased. In the F-5 group, only serum phosphorus was noticeably increased. Hypercalcemia was observed in the MF + D_3 group. In the rats that had received aluminium gel both serum magnesium and phosphorus were lowered.

BUN levels increased in the control group because of a renal infection. BUN levels were significantly lower in the F-2 group and significantly higher in the F-5 group than in the control group. Although renal infections (e.g. infectious hydronephrosis, renal abscess) were frequently noted in the control group, these findings were not as frequent in the F-3 group. Nephrocalcinosis was noted macroscopically in three of ten rats in the F-5 group.

Discussion

Although struvite stones form spontaneously in the urinary tract in specific rat strains [3, 5, 18] stones do not form in rats under normal conditions. On the other hand, struvite stones are easily formed in rats with an experimental urinary tract infection caused by urea-splitting bacteria such as *P. mirabilis* [6, 12, 15] or *Corynebacterium urealyticum* [14]. It is difficult to form calcium stones in rats without nephrocalcinosis [4], which may be attributable to low calcium and high magnesium levels in the rat urine [5].

On the basis of experimental and clinical studies [4, 7, 18], magnesium appears to compete with calcium and plays a role as an inhibitory factor for calcium stone formation. Johansson et al. reported that oral administration of magnesium prevented the recurrence of calcium oxalate stones [7]. We hypothesized that the administration of calcium might inhibit struvite stone formation. The present results show that the high-calcium diet inhibited struvite stone formation, and only small stones composed of calcium carbonate formed. This phenomenon is not attributable to an increase in urinary calcium excretion, but to an obvious decrease in urinary phosphorus, with the result that a large amount of calcium may conjugate with phosphate in the gastrointestinal tract to form an insoluble substance. This assumed mechanism was supported by the findings that administration of vitamin D_3 did not inhibit struvite stone formation while oral administration of aluminium gel did. On the other hand, the low-magnesium diet produced a decrease in urinary excretion of magnesium and inhibited stone formation. Although the high-calcium/high-phosphorus diet indirectly decreased urinary magnesium and inhibited the formation of struvite stones, nephrocalcinosis, probably due to precipitation of calcium phosphate crystals, was noted in some rats.

In order to determine the difference in stone composition between the groups MF, F1 and MF + D_3 and the groups F2, F3, F4, F5 and MF + AL, we studied the architecture of the stones using a scanning electron microscope equipped with an energy-dispersing X-ray

analysis system. Struvite stones formed in rats with urinary tract infection were found to contain minimal calcium compound, probably calcium carbonate (unpublished). Sepcial diets decrease urinary phosphate and/or magnesium and inhibit the precipitation of struvite on zinc discs. As a result, only calcium carbonate can precipitate on the discs.

We have previously reported that the oral administration of some hydroxamic acids, urease inhibitors, prevented struvite stone formation in rats [12, 15]. However, the use of dietary manipulation in the present study was a better inhibitor of stone formation than the urease inhibitors. The extrapolation of the findings of these rat studies to humans should be carried out with caution, because the special experimental conditions cannot be duplicated in humans.

Although urinary pH is one of the most important risk factors for struvite stone formation, patients with urinary tract infections caused by *P. mirabilis* do not always form struvite stones. Urinary phosphate and magnesium may also be important factors. Therefore, diets or drugs decreasing urinary phosphate or magnesium may be useful for the prevention of struvite stone formation. Since an infection stone in humans is composed of struvite and/or carbonate apatite, it may be possible to prevent stone formation by decreasing urinary anions such as the phosphate ion. In practice, some investigators report the effectiveness of aluminium gel on human phosphatic stones [8, 13]. The oral administration of aluminium gel or an anion exchange resin may be useful for the prevention of infection stones, especially those associated with infections that are resistant to various antibiotic regimens.

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