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The effect of verapamil and thiazide in the prevention of renal stone formation

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Summary. The effect of the calcium antagonist verapamil, and of thiazide, a well accepted treatment in the prevention of calcium oxalate renal stones, were examined in an experimental renal stone model. Calcium oxalate stones were induced by the synthetic metabolite of vitamin D_3, the alpha-OH-vitamin D_3 plus ethylene glycol fed rats. A significant decrease in urinary calcium and oxalate was observed following verapamil treatment. Thiazide significantly decreased urinary calcium, but unlike verapamil, did not decrease urinary oxalate. However, no differences in the radiological findings or in the calcium or magnesium content of the kidneys were observed. Although several animal models have been described for the study of calcium oxalate stones, none has yet been proven useful for the evaluation of stone therapy.

Key words: Stones – Calcium channel blockers – Thiazides

Hypercalciuria and hyperoxaluria are important risk factors for kidney stone formation. The high frequency and recurrence rates of calcium oxalate stones make preventative treatment desirable. Although several modalities of treatment have been reported to prevent stones, the results are still controversial. Recently, calcium blockers have been reported to decrease urinary calcium and oxalate excretion in humans [1] and hence these compounds could be of clinical value in stone prevention.

In the present study we have examined the efficacy of verapamil (a calcium blocker) and of thiazide in the prevention of renal stone formation in an experimental stone model. This study shows that the urinary excretion of calcium and oxalate are significantly reduced by verapamil administration. However, neither this drug nor thiazide had any demonstrable effect in the prevention of stone formation when examined by radiography.

Methods

Animal models

An experimental stone model as described by Okada et al. [2] was used. Renal stones were induced by feeding rats 1-alpha-OH-vitamin D_3 (1 – αD3) (0.5 μg every other day) together with ethylene glycol (0.5% added to drinking water) for 14 days. The presence of stones was confirmed by gross examination. Stone analysis by infra red spectroscopy demonstrated calcium oxalate.

Protocol

48 male Wistar rats weight between 200–250 grams were studied. They were randomized into four groups after being adapted to the metabolic cages for one week. All four groups received 0.5% ethylene glycol in the drinking water. In addition, groups II, III and IV also received 0.5 μg 1-αD_3 by mouth every other day. Group III was treated with verapamil (10 mg/kg/day i.p.) and group IV received chlorothiazide (2 mg/kg/day i.p.). During the studies the rats received a diet containing magnesium 0.21% (as magnesium sulphate), calcium 1.01% (as elemental calcium), phosphorus 0.74% as (K_2HPO_4) and water ad lib.

Clearance methods

Several 24 hour clearance studies were performed in all experimental animals with the aid of metabolic cages. Body weight and fluid intake were monitored. Free flowing tail vein blood was collected from each animal once weekly for the determination of electrolyte concentrations, creatinine and blood urea nitrogen. The following parameters were measured from the collected urine for the duration of the study: urine volume, sodium, potassium, calcium, magnesium, phosphate, creatinine, citrate, oxalate, and hydroxyproline. Endogenous creatinine clearance were used to determine the glomerular filtration rate.

At the end of the experiment the rats were sacrificed by decapitation and the kidneys were quickly removed and dried for 2 days in a vacuum oven at 95 °C in the presence of KOH. Kidneys were then examined by x-ray for radio opaque stones. The kidneys were then ground into a fine powder with a mortar and pestle and dried in a vacuum at 95 °C overnight. 200 mg of dried kidney tissue was dissolved in 1 ml of concentrated nitric acid for the deter-

Table 1. Summary of clearance data obtained from all four groups of animals examined prior to and following the experiment

Group	I		II		III		IV	
Urine	(EG)		(EG + Vit D_3)		(EG + Verapamil + Vit D_3)		(EG + CTZ + Vit D_3)	
	B $n=12$	A $n=12$	B $n=12$	A $n=12$	B $n=12$	A $n=12$	B $n=12$	A $n=12$
Urine volume ml/min	0.016±0.004	0.022±0.004	0.017±0.004	0.056±0.011[b]	0.014±0.002	0.050±0.012[b]	0.013±0.001	0.048±0.009[b]
GFR ml/min	1.85±0.04	2.68±0.08[b]	2.01±0.11	1.80±0.12	2.0 ±0.07	1.58±0.15[c]	2.07±0.10	1.54±0.14[c]
FE Na %	0.67±0.01	0.60±0.01	0.67±0.02	0.79±0.02[b]	0.62±0.1	0.77±0.04[b]	0.63±0.01	0.80±0.07[c]
FE K %	44.32±1.08	34.89±1.02[b]	40.72±0.92	41.95±1.25	39.47±1.0	44.53±2.0[c]	38.0±1.5	45.0 ±1.4[c]
FE Ca %	1.23±0.11	0.99±0.12[c]	1.51±0.23	4.40±0.77[b]	1.71±0.04	3.38±0.42[a]	1.67±0.19	3.24±0.50
FE Mg%	22.18±1.21	21.77±1.19	19.8 ±1.0	34.0 ±1.57[b]	18.51±0.57	34.82±2.36[b]	27.17±0.71	37.23±2.1[b]
FE Pi %	12.42±0.52	12.62±0.61	12.07±0.51	17.94±1.73[b]	12.22±0.35	17.12±1.48[c]	10.32±0.64	20.01±1.01
Citrate mmol/d	0.351±0.021	0.463±0.018	0.441±09.030	0.448±0.030	0.401±0.028	0.388±0.035	0.418±0.018	0.365±0.066
Hydroxy proline mmol/d	13.0 ±0.4	12.2 ±0.6	12.2 ±0.3	9.8 ±0.5	12.5 ±0.2	8.2 ±0.4	12.2 ±0.2	7.8 ±1.0[a]
Oxalate mmol/d	61.1 ±2.8	62.2 ±0.3	59.4 ±0.3	66.4 ±7.8	61.1 ±2.2	33.9 ±2.3	60.0 ±0.3	99.1 ±9.3[a]
BLOOD								
PCa mmol	2.5 ±0.03	2.6 ±0.01	2.5 ±0.01	2.91±0.04[b]	2.5 ±0.01	2.8±0.04[b]	2.54±0.02	2.87±0.06
BUN mg%	21.0 ±0.03	24.0 ±0.72	21.5 ±0.89	33.1 ±1.37[b]	20.48±0.75	33.8 ±2.3	18.7 ±0.60	39.91±4.81

GFR = glomerular filtration rate; FE = fractional excretion; P_{Ca} = plasma calcium; BUN = blood urea nitrogen; B = Control; A = 18 days after treatment; GE = ethylene glycol

[a] <0.001 as compared to groupII; [b] <0.001 as compared to each baseline period; [c] <0.05 as compared to each baseline period

mination of calcium and magnesium. Calcium and magnesium were measured in the supernatant by atomikc absorption spectrophotometry.

Analytical methods

Chemical analyses included sodium and potassium by flame photometry (943 photometer), calcium and magnesium (atomic absorption, Jarell As 850), and phosphate by the molybdate method. Blood urea nitrogen and creatinine were measured by the Urease and Jaffee methods respectively. Urinary oxalate and hydroxyproline were estimated by high performance liquid chromatography [8, 9] respectively. Urinary citgrate was quantitated by high performance ion chromatography [17].

X-ray

Dried kidneys were examined in a Fax Itron oven 4380N (Hewlett Packard) at a dose of 20 Kv for 40 seconds.

Statistical analyses were performed by analysis of variance and paired "t" test when appropriate.

Results

The clearance results are shown in Table 1. There was no significant difference in the fractional excretion of sodium, magnesium, potassium, phosphorus, and 24 hour urinary excretion of citrate in groups III and IV as compared to group II. Absolute and fractional urinary excretion of calcium (Fig. 1) was significantly decreased in groups III and IV when compared to group II (3.38 ± 0.42, 3.24 ± 0.50 vs. $4.40\pm0.77\%$, $p<0.001$) respectively. Urinary oxalate (Fig. 2) was augmented in group IV and decreased in group III in comparison with group II (8.92 ± 0.84, 3.05 ± 0.21 vs. 5.98 ± 0.70 mg/24 h, $p<0.001$) respectively. Groups III and IV also had a significant reduction in GFR.

Radiological examination in a Faxitron oven at a dose of 20 Kv for 40 seconds showed the presence of stones in groups II, III and IV. Infrared spectrophotometric analysis confirmed that these stone consisted of calcium oxalate.

No significant difference was found in the calcium or magnesium content of the kidneys between groups III and IV (Ca 39.4 ± 5.2 vs 39.2 ± 3.1, Mg 1.18 ± 0.20 vs. 1.38 ± 0.30 mg/g DW kidney pNS) respectively.

Discussion

Goligorsky et al. [5] reported that verapamil inhibited nephrocalcinosis, preventing mitochondrial and tubular basement membrane calcification in a subtotal nephrec-

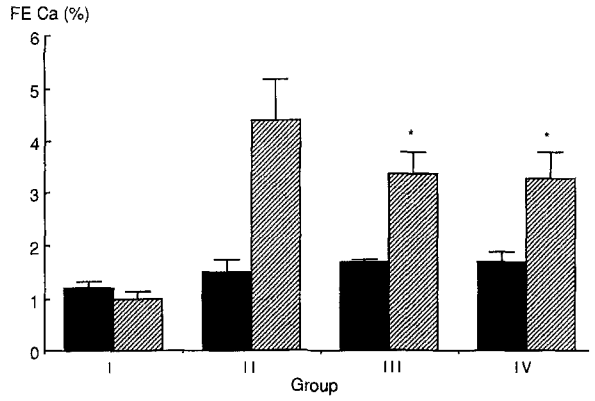

Fig. 1. The effect of verapamil treatment on fractional excretion of calcium in Groups I, II, and IV. + Before treatment; ▨ after treatment; * $p<0.001$ compared to group II

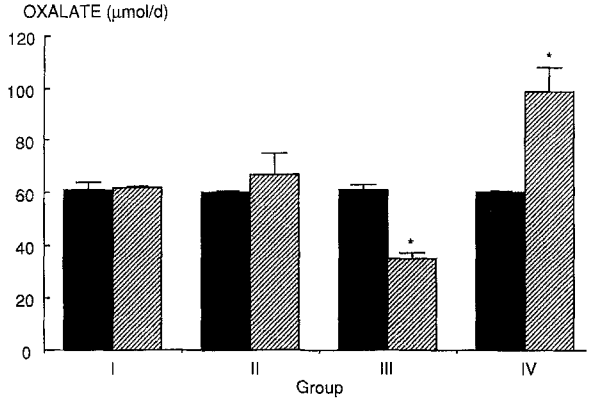

Fig. 2. The effect of verapamil treatment on oxalate excretion in Groups I, II, III and IV. + Before treatment; ▨ after treatment; * $p<0.001$ compared to group II

tomy model of renal failure after 3 weeks of treatment. Harris et al. [6] utilizing the same experimental model for 15 weeks, demonstrated that verapamil protects against renal failure, histological damage, nephrocalcinosis and myocardial calcification. On the other hand, Bichler et al. [2] studied the effect of nifedipine (Adalat) administration in an animal model of nephrocalcinosis induced by an atherogenous diet. They reported an increase in the urinary calcium and sodium excretion while urinary phosphate and potassium levels were lowered. No effect on oxalate metabolism was reported. The authors suggested that hypercalciuria was a result of a reduced calcium reabsorption in the tubules and suggested that the calcium antagonist might have inhibited the calcium influx into the tubular cells. Baggio et al [1] reported that nifedipine decreases urinary calcium and oxalate excretion in man. A year later, Gambaro et al. [3] reported that flunarizine, another calcium antagonist reduced the urinary oxalate excretion in humans without any significant effects on urinary calcium excretion. Since the results of these studies were inconsistent, we examined the role of the calcium antagonist verapamil on calcium and oxalate metabolism. We also compared the effect of verapamil with that of thiazide, a well accepted agent, for the prevention of stone formation. The results of our study indicate that verapamil significantly decreases urinary calcium and oxalate excretion without preventing stone formation. Thiazides were proposed for calcium stone prevention because of their hypocalcuric action [4, 11, 12]. The hypocalcuric effect of thiazides is present after 6 days of treatment and is sustained for as long as the drug is taken. In our study, thiazides did significantly reduce the renal excretion of calcium.

The long term effect of thiazides on urinary oxalate excretion is controversial. Yendt [19] and Scholz [16] reported a decline in urinary oxalate excretion after one year of treatment and suggested that this may have been the result of reduced urinary oxalate excretion or the decrease in intestinal calcium absorption. Unlike these authors, we have observed a significant increase in urinary oxalate in the thiazide group which may have been due to a short period of treatment.

Urinary citrate excretion has been reported to decrease after one month of hydrochlorothiazide treatment [12]. This effect of thiazide on urinary citrate has also been noted by Garcia et al. [4]. They attributed the effect to hypokalemia or possibly magnesium depletion. In our study, no significant difference was found in the urinary citrate or in the fractional excretion of magnesium or potassium between the groups.

A change in plasma potassium concentration is a common complication of diuretic therapy. Plasma potassium tends to correlate inversely with the dose of the diuretics [13] and the duration of therapy [14]. Diuretics with the longest duration of action such as chlorthalidone produces marked hypokalemia and not hyperkalemia as observed in groups III and IV (5.53 ± 0.31 vs. 5.46 ± 0.40 mmol/l NS). Hyperkalemia can be observed when using potassium sparring diuretics or during simultaneous administration of diuretics and potassium salts. Hyperkalemia can also be present as a result of a reduction in renal function. In our study we have used a thiazide (chlorothiazide) that usually produced hypokalemia. The slight hyperkalemia observed in our study may be due to a decrease in renal function in groups III and IV.

Bone turnover has been reported to decrease with thiazide therapy in humans [7] and a decreasing urinary hydroxyproline excretion has been observed in humans [18] and in the rat [10] during chronic thiazide administration. We have observed a significant difference in urinary hydroxyproline excretion in group III and IV as compared to group II. In summary, the results of this study show that both verapamil and thiazide have significant effect on urinary calcium or oxalate excretion. However, no beneficial effect was noticed in the prevention of renal stone formation or in the calcium or magnesium content of the kidney in either group. It appears that this model is too sensitive for the evaluation of chronic potential inhibitors of stone formation.

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