Urol Res (1994) 22: 301–303

ORIGINAL PAPER

W. L. Strohmaier · R. D. Seeger · H. Osswald
K. H. Bichler

Reduction of vitamin D induced stone formation by calcium

Received: 9 June 1994 / Accepted: 10 August 1994

Abstract Investigations were carried out as to whether cytoprotective agents such as calcium antagonists can influence vitamin D induced nephrolithiasis. Increased vitamin D levels are found in 10–30% of all calcium oxalate stone formers. Male rats were assigned to one of the following groups: (1) 1,25-dihydroxycholecalciferol (DHCC) ($n=8$), (2) 1,25-DHCC + calcium antagonist Goe 6070 (a new 1,4-dihydronaphthyridine, Goedecke, Berlin) ($n=8$), or (3) control ($n=8$). 1,25-DHCC was administered for 6 days (120 pmol/24 h s.c.), Goe 6070 (1 mg/kg/24 h) by gavage. Clearance studies were performed on day 6. Kidneys were taken for histological examination and determination of calcium tissue content. 1,25-DHCC induced substantial concrement formation, which could be significantly limited by Goe 6070. The calcium tissue content was also reduced (0.17 vs. 0.04 mg/100 mg dry weight). 1,25-DHCC induced a dramatic fall in the glomerular filtration rate (GFR) (3.84 ml/min per kilogram). This reduction could be almost completely inhibited by the concomitant application of Goe 6070 (9.4 ml/min per kilogram; control 10.7 ml/min per kilogram). Goe 6070 did not influence the calcium handling. The results demonstrate a protective effect of Goe 6070 on vitamin D induced nephrolithias. The histological pattern (intracellular and membrane-bound concretions) and the fact that biochemical parameters were not influenced significantly by Goe 6070 indicate that cellular processes are important for 1,25-DHCC-induced nephrolithiasis.

Key words Vitamin D · 1,25-Dihydroxycholecalciferol
Nephrolithiasis · Calcium antagonists

W. L. Strohmaier (✉) · R. D. Seeger · K. H. Bichler
Department of Urology, Eberhard-Karls University,
Hoppe-Seyler-Strasse 3, D-72076 Tübingen, Germany,
Fax: +49(70 71) 29 50 92

H. Osswald
Institute of Pharmacology, Eberhard-Karls University,
Wilhelmstrasse 56, D-72074 Tübingen, Germany

1,25-Dihydroxycholecalciferol (DHCC), the active metabolite of vitamin D, plays an important role in the pathogenesis of calcium urolithiasis. Increased 1,25-DCHH levels are found in 10–30% of all calcium oxalate stone formers [1, 3, 5, 9, 13]. The underlying mechanisms, however, are far from being completely understood. Most frequently stone formation was attributed to hypercalcemia and hypercalciuria with consequent supersaturation of the urine [1, 9]. Scarpelli et al. [10], however, explained it by vitamin D induced renal epithelial damage and cellular calcification as the starting point of concrement formation. In an experimental study we investigated whether cellular processes are important for the pathogenesis of vitamin D induced nephrolithiasis. Assuming this hypothesis to be true cytoprotective agents such as calcium antagonists should influence these processes.

Materials and methods

Male Wistar rats (body weight 250–300 g, Chbb: THOM, Thomae, Biberach/R., Germany) were randomly assigned to one of the following groups: (1) 1,25-DHCC ($n=8$), (2) 1,25-DHCC plus calcium antagonist Goe 6070 (Goedecke, Berlin, Germany) ($n=8$), or (3) control ($n=8$). 1,25-DHCC (Roche, Basle, Switzerland) was administered for 6 days (120 pmol/24 h s.c.), Goe 6070 for 6 days (1 mg/kg body weight per 24 h) by gavage. Goe 6070, a new 1,4-dihydronaphthyridine currently under development, is a high-affinity specific L-type voltage-gated calcium channel blocker [6–8]. Its half-life of >12 h enables an application to be made once a day. Clearance studies (inulin, creatinine, calcium, phosphate) were performed on day 6 as described recently [14]. The fractional excretion (FE) of calcium and phosphate was calculated [e.g., FE Ca (%) = calcium clearance (ml/min)/inulin clearance (ml/min) × 100]. The kidneys were taken for the measurement of calcium tissue concentration and the histological examination (Kossa and Voigt staining) with determination of the calcification index (CI) [2]. The CI is a semiquantitative grading of renal calcification (– no calcifications, 1+ low, 2+ moderate, 3+ strong). This analysis was done by three independent coworkers who did not know to which group the specimens belonged or what the analysis of their coworkers was. The average CI was calculated for each group using the predetermined calcification grades. For statistical analysis, Student's t-test was used for a Gaussian distribution and the same variance. Parameters with a non-Gaussian distribution and/or different variances were analyzed by the Wilcoxon-Mann-Whitney test.

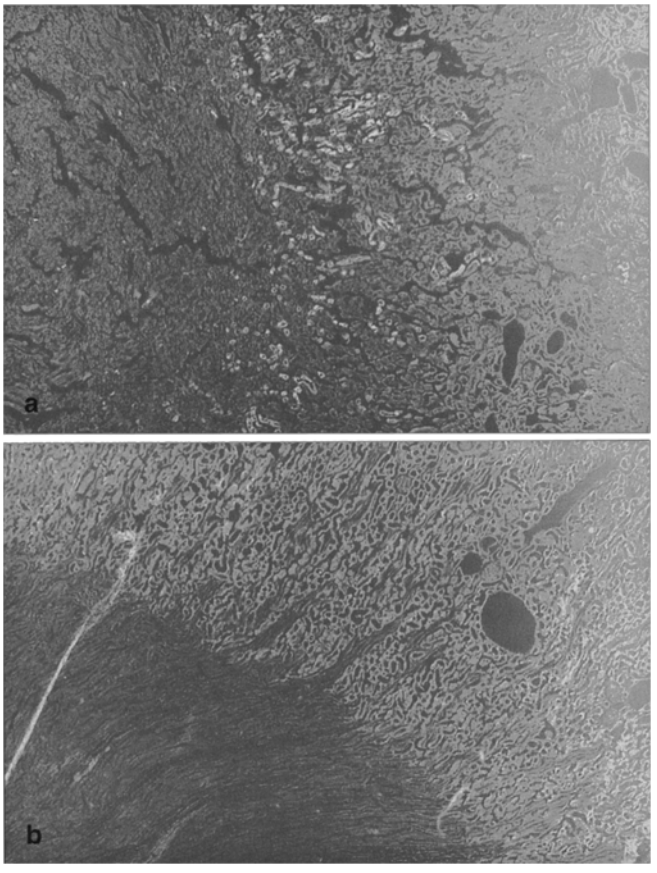

Fig. 1 a, b Longitudinal sections of the kidney. Kossa stain, ×25. Calcification were predominantly located in the corticomedullary junction zone. **a** 1,25-DHCC, **b** 1,25-DHCC + Goe 6070

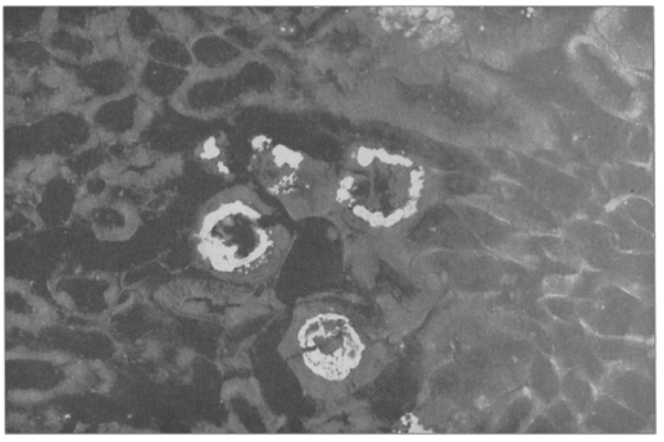

Fig. 2 Intracellular and membrane standing calcifications in renal tubules of the corticomedullary junction zone. Kossa stain, ×400

Table 1 Mean values ±SD of plasma calcium and phosphate concentrations and of the histological calcification index (*CI*) and the calcium content of renal tissue of 1,25-DHCC, 1,25-DHCC + Goe 6070 and control groups (significant differences vs. control: [a] $P<0.01$; [b] $P<0.05$; 1,25-DHCC vs. 1,25-DHCC + Goe 6070: [c] $P<0.01$)

	Calcium (mmol/l)	Phosphate (mmol/l)	CI	Ca content (mg/100 mg dry weight)
1,25-DHCC	2.27 ± 0.19[a]	2.46 ± 0.27[b]	2.40 ± 0.82[a,c]	0.1680 ± 0.0857[a,c]
1,25-DHCC + Goe 6070	2.47 ± 0.20[a]	2.51 ± 0.25[b]	1.45 ± 0.82[a]	0.0433 ± 0.0214[a]
Control	1.91 ± 0.20	2.08 ± 0.29	0.36 ± 0.38	0.0188 ± 0.0036

Results

1,25-DHCC induced substantial concrement formation, which was significantly less pronounced in the 1,25-DHCC plus Goe 6070 group (Table 1). The microliths were not homogeneously distributed all over the kidney, but concentrated predominantly in the area of the corticomedullary junction (Fig. 1). Concrements were located within the tubular cells or were bound to the luminal membrane (Fig. 2). Free intraluminal particles could be observed only rarely and there were no signs of relevant tubular obstruction. 1,25-DHCC rats showed significantly higher calcium tissue concentrations in the kidney than 1,25-DHCC plus Goe 6070 animals (Table 1). The glomerular filtration rate (GFR) was significantly reduced by 1,25-DHCC. This could be almost completely inhibited by concomitant application of Goe 6070 (Table 2). 1,25-DHCC anmials showed increased plasma calcium (Table 1) and fractional excretions of calcium and phosphate (Table 2). These parameters were not changed by Goe 6070. Plasma phosphate levels were not statistically different between the study groups (Table 1).

Table 2 Mean values ±SD of inulin and creatinine clearance and of fractional excretions (*FE*) of calcium (*Ca*) and phosphate (*P*) of the 1,25-DHCC, 1,25-DHCC + Goe 6070 and control groups. Significant differences vs. control: [a] $P<0.01$, [b] $P<0.05$, 1,25-DHCC vs. 1,25-DHCC + Goe 6070: [c] $P<0.01$

	Inulin (ml/min/kg)	Creatinin (ml/min/kg)	FE Ca (%)	FE P (%)
1,25-DHCC	3.84 ± 1.69[a,c]	4.20 ± 1.05[a,c]	3.48 ± 2.60[a]	19.6 ± 10.7[b]
1,25-DHCC + Goe 6070	9.39 ± 1.59	9.59 ± 1.47	1.96 ± 1.28[b]	16.8 ± 7.3[b]
Control	10.69 ± 0.93	9.88 ± 1.92	0.64 ± 0.28	9.1 ± 3.6

Discussion

Our results demonstrate that 1,25-DHCC induced a marked concrement formation in the corticomedullary

junction area, which starts within the tubular cells. The corticomedullary junction is a predisposed area for concrement formation in the rat kidney, since the epithelium of this zone is highly differentiated and there is a high concentration of minerals (tip of the loop of Henle), which can result in supersaturation [12].

This supersaturation with calcium could easily explain the concrement formation. The calcium antagonists treated group, however, revealed significantly less calcifications, although the calcium plasma and urine concentrations were similar to those in the animals which received only 1,25-DHCC. Therefore we assume that cellular factors are important for the initiation of 1,25-DHCC-induced concrement formation. Scarpelli et al. [10] have already discussed cellular damage as a cause of vitamin D nephrolithiasis, since after vitamin D administration a vacuolization in the apical parts of the tubular cells (i.e., a sign of cell damage) preceded the occurrence of calcifications by several days. High doses of vitamin D inhibited the mitochondrial calcium accumulation and the oxidative phosphorylation which results in cytoplasmic supersaturation with calcium [10]. In the absence of ATP, apatite and brushite are formed [17].

The fact that the calcium antagonist Goe 6070 limited the concrement formation supports the theory of cellular initiation of 1,25-DHCC-induced nephrolithiasis, since calcium entry blockers are able to inhibit the calcium overload in renal tubular cells [11]. Figure 3 summarizes this theory of vitamin D induced nephrolithiasis and the beneficial role of calcium antagonists.

The GFR was dramatically reduced by 1,25-DHCC. Since no relevant signs of tubular obstructions could be observed, the reduced GFR may be attributed to an 1,25-DHCC-induced increase in the contractility of mesangial cells. Weinreich et al. recently demonstrated that 1,25-DHCC enhances the contractility of mesangial cells [16]. This action results in a decreased glomerular filtration surface area and consequently in a reduced GFR. A second reason for the decreased GFR could be a hypercalcemia-induced increase in the resistance of the vas afferens [15].

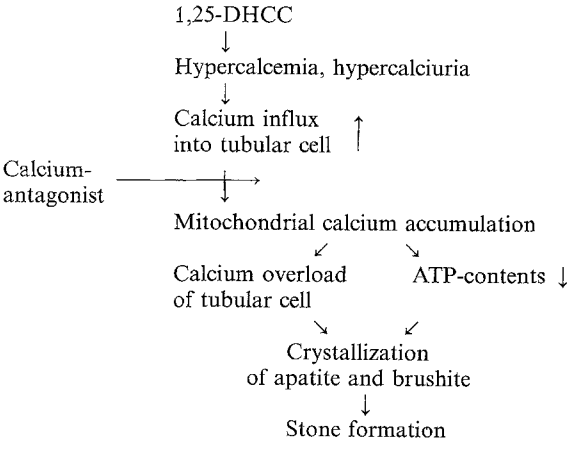

Fig. 3 Theorey of 1,25-DHCC-induced renal stone formation and the beneficial effect of calcium antagonists

The concomitant application of Goe 6070 almost completely inhibited the reduction of the GFR. This may be explained by an inhibitory effect of calcium antagonists on mesangial cell contraction or on vascular smooth muscle contraction [4].

Acknowledgements We thank Dr. G. Satzinger, Goedecke AG, Berlin, Germany for the generous gift of Goe 6070 and Dr. H. Weisser, Roche, Basle, Switzerland for providing us with 1,25-DHCC.

References

1. Baumann JM (1980) Pathophysiologie und medikamentöse Therapie der Phosphat-Stein-Erkrankung. In: Vahlensieck W (Hrsg) Urolithiasis, vol 4. Springer, Berlin Heidelberg New York, S 4
2. Bichler KH, Strohmaier WL (1988) Nephrocalcinosis, calcium antagonists and kidney. Springer, Berlin Heidelberg New York
3. Bichler KH, Strohmaier WL, Schreiber M, Korn S, Gaiser I (1984) Hyperkalziurie und Vitamin-D-Stoffwechsel. Fortschr Urol Nephrol 22:342
4. Boonen HCM, DeMey JGR (1990) Increased calcium sensitivity in isolated resistance arteries from spontaneously hypertensive rats. Effects of dihydropyridines. Eur J Pharmacol 179:403
5. Insogna KL, Broadus AE, Dreyer BE, Ellinson AS, Gertner JM (1985) Elevated production rate of 1,25-dihydroxyvitamin D in patients with absorptive hypercalciuria. J Clin Endocrinol Metab 61:490
6. Kleinschroth J, Hartenstein J, Mergelsberg I, Osswald H, Steinbrecher W, Wagner B, Weinheimer G, Satzinger G (1988) Novel 1,4-dihydro-1,6-naphthyridines as antihypertensive calcium antagonists. Abstr 10th Int Symp Med Chem Budapest
7. Osswald H, Steinbrecher W, Weinheimer G, Wagner B, Kleinschroth J, Hartenstein J, Satzinger G (1988) Antihypertensive activity of a novel calcium antagonist. Goe 5584-A, in rat models of hypertension. J Cardiovasc Pharmacol 12II [Suppl 6]:154
8. Osswald H, Weinheimer G, Kapp JF (1990) Renal actions of calcium channel antagonists. J Neural Transm 31 [Suppl]:39
9. Pak CYC (1978) Hypercalciurias. In: Pak CYC (ed) Calcium urolithiasis. Plenum Medical Book Co, New York, p 37
10. Scarpelli DG, Tremblay G, Pearse AGE (1960) A comparative cytochemical and cytological study of vitamin D induced nephrolithiasis. Am J Pathol 36:331
11. Schrier RW, Arnold PF, van Putten VJ, Burke TJ (1987) Cellular calcium in ischemic acute renal failure: role of calcium entry blockers. Kidney Int 32:313
12. Schwille PO, Brandt P, Ulbrich D, Kömpf W (1975) Pankreasinseln, Plasmaglukagon und renale Verkalkungen unter verschiedener Grunddiät bei der Ratte. Urologie A 14:306
13. Stapleton FB, Langman CB, Bittle J, Miller LA (1987) Increased serum concentrations of 1,25(OH)2 vitamin D in children with fastig hypercalciuria. J Pediatr 110:234
14. Strohmaier WL, Seeger D, Osswald H, Bichler KH (1992) Renal insufficiency induced by parathyroid hormone: influence of the calcium antagonist Goe 6070. Eur Urol 21:323
15. Thier SO (1978) Renal insufficiency and hypercalcemia. Kidney Int 14:194
16. Weinreich T, Merke J, Schönermark M, Reichel H, Diebold M, Haensch GM, Ritz E (1991) Actions of 1,25-dihydroxyvitamin D3 on human mesangial cells. Am J Kidney Dis 18:359
17. Wuthier RE, Rice GS, Wallace JEB, Weaver RL, LeGeros RZ, Eanes ED (1988) In vitro precipitation of calcium phosphate under intracellular conditions: formation of brushite from an amorphous precursor in the absence of ATP. Calcif Tissue Int 41:401