Effects of 1,25-Dihydroxyvitamin-D₃ Treatment on Mineral Balance in Children with End Stage Renal Disease Undergoing Chronic Hemofiltration

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ABSTRACT. Ten children with end stage renal disease on chronic hemofiltration (HF) were studied for a 1-yr period to evaluate the efficacy of 1,25-dihydroxyvitamin-D₃ (1,25(OH)₂D₃) therapy on biohumoral parameters of renal osteodystrophy and bone mineral content. In six of these children an acute study was done of the direct effect of the HF procedure on calcium and phosphate balance during 12 HF sessions. During the first 6 months of the study all children were treated with 1,25(OH)₂D₃ (0.25–0.50 µg/day) to maintain plasma calcium at 9.5–11.0 mg/dl. There was a significant increase in plasma calcium ($p < 0.05$) and a significant decrease in plasma phosphate ($p < 0.01$) and alkaline phosphatase concentrations ($p < 0.05$). The circulating levels of NH₂ immunoreactive parathyroid hormone did not change, remaining at the upper limits of reference values. Immunoreactive parathyroid hormone-COOH terminal fragment levels decreased significantly ($p < 0.05$). Bone mineral content rose significantly ($p < 0.01$). During the last 6 months of the study, to evaluate the possibility that HF alone might control secondary hyperparathyroidism, 1,25(OH)₂D₃ treatment was discontinued in five children; plasma calcium and phosphate were well controlled whereas hyperparathyroidism worsened in all five, and one also developed intense pruritus and hypertension. The other five children remained on 1,25(OH)₂D₃ treatment; two of these were transplanted, and the other three continued to show an improvement of mineral balance. The results of the acute study showed that calcium balance was positive with a mean Ca⁺⁺ gain of 140 mg/HF session. The mean total phosphate removed per HF run was 574 mg. We conclude that even though a calcium gain and a high phosphate removal was obtained with our HF prescription, secondary hyperparathyroidism in children on chronic HF may be maintained under control if 1,25(OH)₂D₃ supplementation is provided. (*Pediatr Res* 20: 5–8, 1986)

Abbreviations

BMC, bone mineral content
ESRD, end stage renal disease
HD, hemodialysis
HF, hemofiltration
iPTH, immunoreactive parathyroid hormone
1,25(OH)₂D₃, 1,25-dihydroxyvitamin-D₃

There are many reports of long-term 1,25(OH)₂D₃ treatment of children with ESRD with and without chronic HD or chronic ambulatory peritoneal dialysis (1–7). However, there has been no study of such treatment in children on chronic HF, a replacement method based on convective transport (8) recently introduced in children (9). This method has been reported to be more effective than HD in removing toxins of middle and large molecular weight (10) and in reducing hypotension and other symptoms of dialysis intolerance in uremic adults and children (9, 11–13). HF has been variously claimed to improve (13), worsen (14), or not change (15) hyperparathyroidism in uremic adults. Such discrepancies may be explained by differences both in vitamin D supplementation and in calcium balance during the HF procedure. The aim of the present study was to investigate the effect of 1,25(OH)₂D₃ treatment on the most important biohumoral parameters of renal osteodystrophy and BMC in 10 children on chronic HF. In addition, we evaluated mineral metabolism in five of these children on HF without vitamin D supplementation. Finally, an acute study was done of the effect of the HF procedure on calcium and phosphate balance during 12 HF sessions.

MATERIALS AND METHODS

Patients. Ten children (age 5.9–15.0 yr) with ESRD on chronic HF were prospectively studied for 1 yr after treatment by HD for a mean of 26.6 months (range 9–90 months) (Table 1). All were anuric at the start of the study, except patient 3 who had a residual renal glomerular function below 1 ml/min. Two patients were nephrectomized 3 yr earlier because of uncontrolled hypertension. No calcium or phosphate supplementation was prescribed during the study. Systemic acidosis was corrected with oral bicarbonate therapy, both before and during the study. Reference values for plasma calcium, phosphate, and alkaline phosphatase levels were obtained from a control group of 110 normal children (age 5–15 yr). Reference values of BMC, iPTH, and vitamin D metabolites were available from 60 of the control children.

STUDY PROTOCOL

Long-Term Study. The 12-month study was divided into two periods of 6 months each as follows.

Period I. All the children, previously on HD, started HF. During the last 6 months of HD, 1,25(OH)₂D₃ (0.25–5.0 µg/day) was introduced to maintain plasma calcium at 9.5–11.0 mg/dl. The children continued 1,25(OH)₂D₃ treatment during HF.

Period II. All patients remained on HF but were divided at

Table 1. Patient characteristics

Patient	Sex	Age (yr)	Duration of HD (mo)	Diagnosis
1	F	5.9	9	Acroosteolysis
2	F	6.9	18	Cystinosis
3	M	7.0	9	Nephrotic syndrome (focal segmental sclerosis)
4	M	8.0	16	Unknown
5	M	13.8	18	Nephronophthisis
6	F	5.9	25	Congenital nephrotic syndrome
7	M	10.9	42	Hemolytic uremic syndrome
8	F	11.0	18	Hemolytic uremic syndrome
9	M	6.9	9	Nephrotic syndrome (focal segmental sclerosis)
10	M	15.0	90	Endoextracapillary glomerulonephritis

random into subgroups of five children each. Subgroup 1 continued to take $1,25(\text{OH})_2\text{D}_3$; vitamin D was discontinued in subgroup 2 children. Two of the children in subgroup 1 were transplanted during this period and these children were not studied further. Patient 4 developed manifestations of severe hyperparathyroidism and was studied for an additional 3 months.

Acute Study. To evaluate the effects of HF per se on mineral balance, we studied calcium and phosphate balance during an HF session twice in each of six children for a total of 12 balances. Ultrafiltrate samples consisting of the fluid obtained in 1 min were drawn every half-hour. The calcium balance was determined according to the formula:

$$\text{Ca}_B = \text{Ca}_{\text{SF}} \times \text{IR} - \text{Ca}_{\text{UF}} \times \text{UR}$$

where Ca_{SF} is the substitution fluid calcium concentration (mg/dl), IR is the fluid reinfusion rate (ml/min), Ca_{UF} is the ultrafiltrate calcium concentration (mg/dl), and UR is the ultrafiltration rate (ml/min). Blood samples were drawn at the beginning and at 1-h intervals until the end of the session. In all the samples plasma calcium and phosphate were measured. In addition we measured the hematocrit and plasma protein concentration in the first and last samples.

METHODS

HF was performed with a hollow-fiber polyamide membrane determining a mean ultrafiltration rate of 66.7 ± 15.0 ml/min, with a mean blood flow of 210 ± 69 ml/min. The replacement of the substitution fluid ($\text{Na}^+ = 140$; $\text{K}^+ = 1.5$; $\text{Cl}^- = 105$; $\text{Mg}^{++} = 1.5$; $\text{Ca}^{++} = 4.5$; lactate = 42 mEq/liter) was regulated employing a postdilutional computerized method using the gravimetric principle. HF was prescribed to maintain a pretreatment blood urea nitrogen value of 100 ± 20 mg/dl by a urea kinetic model (16).

Plasma iPTH was measured by radioimmunoassay using a specific chicken anti-iPTH serum directed against 1–34 human PTH (NH_2 -iPTH) and a second antiserum directed against 65–84 human PTH (COOH -iPTH). For both assay systems the intraassay coefficient of variation obtained in our laboratory was 3%; the interassay variation was 7–8% in accordance with data in the literature (17). Plasma vitamin D metabolites were extracted with diethyl ether; 25-hydroxycholecalciferol and $1,25(\text{OH})_2\text{D}_3$ were separated and purified with three elutions in a silicic acid column. Metabolites were further characterized by high-protein liquid chromatography. 25-Hydroxycholecalciferol and $1,25(\text{OH})_2\text{D}_3$ were measured by radioimmunoassay (18, 19). Our intra- and interassay variations were 4 and 9%, respectively, for both methods.

BMC was evaluated by direct photon absorptiometry with a Gambro Bone Densitometer (^{125}I source) connected on-line with

an Apple II computer. Measurements were made at midshaft of the radius and ulna of the nonfistula forearm. Roentgenograms of hands, knees, ankles, shoulders, pelvis, hips, lumbar spine, and skull were performed in all the children a month before beginning the study. Skeletal radiography was repeated at the end of the study only in the patients who showed radiological signs compatible with secondary hyperparathyroidism at the first radiography.

Data are presented as means $\pm$ SD. Data were analyzed with Student's *t* test for paired data for the first period of the study. For the second period only clinical observations and mean values or percentages are presented because of the small number of patients in the two subgroups.

Informed consent was obtained from patients' parents and individually from the older children.

RESULTS

LONG-TERM STUDY

Period I. Biohumoral parameters obtained immediately before beginning HF and after the first 6 months of $1,25(\text{OH})_2\text{D}_3$ treatment are shown in Table 2. After 6 months of HF and $1,25(\text{OH})_2\text{D}_3$ treatment there was an improvement of some of the biochemical parameters considered. Plasma calcium increased significantly ($p < 0.05$). Hypercalcemia (>11.0 mg/dl) was observed in 11 of 120 measurements (9%) made during the period. Plasma phosphate levels decreased significantly ($p < 0.01$). There were 120 plasma phosphate measurements for this period; one episode of hypophosphatemia and 17 episodes (14% of all the determinations) of hyperphosphatemia were observed (compared with reference values obtained in our normal children which differ according to age). The plasma calcium $\times$ phosphate product exceeded 60 in only 6% of determinations. The mean dose of oral aluminum hydroxide to maintain plasma phosphate in the normal range for age decreased significantly during the period (from 121 ± 46 mg/kg/day at the beginning of the study to 88 ± 54 mg/kg/day at month 6; $p < 0.02$). Mean values for plasma alkaline phosphatase and iPTH-COOH decreased significantly ($p < 0.05$); mean iPTH- NH_2 remained unchanged as did mean 25-hydroxyvitamin- D_3 and $1,25(\text{OH})_2\text{D}_3$. At the beginning of the study 4 of the 10 children studied had BMC 3 SD below the mean of our normal children of the same age and sex; another child had a BMC below 2 SD; in two it was under 1 SD, and only three children had BMC in the normal range for sex and age. BMC rose significantly in all the children during this period (radius: 383 ± 26 mg/cm 2 at the beginning versus 404 ± 27 mg/cm 2 at month 6; $p < 0.01$ (Fig. 1); ulna: 385 ± 88 versus 417 ± 82 mg/cm 2 ; $p < 0.01$). The skeletal radiographic evaluations performed in all children 1 month before beginning the study were normal in all but patient 6 who had a cyst in the proximal region of one femur compatible with secondary hyperparathyroidism.

Period II. Subgroup 1. Mean plasma calcium phosphate levels

Table 2. Biohumoral findings in 10 uremic children at baseline (T_0) and after 6 mo (T_6) of HF and $1,25(\text{OH})_2\text{D}_3$ therapy (values expressed as means $\pm$ SD)

	T_0	T_6	<i>p</i>
Calcium (mg/dl)	10.0 ± 0.6	10.3 ± 0.6	<0.05
Phosphate (mg/dl)	4.9 ± 0.6	4.2 ± 0.3	<0.01
Magnesium (mg/dl)	2.5 ± 0.6	2.5 ± 0.3	NS
Alkaline phosphatase (U/liter)	85 ± 50	77 ± 44	<0.05
iPTH-COOH (pg/ml)	2035 ± 1375	1765 ± 1138	<0.05
iPTH- NH_2 (pg/ml)	252 ± 129	266 ± 183	NS
25-Hydroxyvitamin- D_3 (ng/ml)	46 ± 22	39 ± 16	NS
$1,25(\text{OH})_2\text{D}_3$ (pg/ml)	23 ± 9	24 ± 9	NS

remained unchanged during the period for the three children treated with $1,25(\text{OH})_2\text{D}_3$ (10.4 ± 0.3 and 4.2 ± 0.4 mg/dl, respectively). Oral aluminum hydroxide dosage was not changed in any of the patients. In all three children there was a further decrease in alkaline phosphatase and iPTH (Fig. 2) and an increase in BMC (Fig. 1). At the end of the period the skeletal radiography was repeated in patient 6; there was no change in the cyst compared with the previous roentgenogram and no other signs of secondary hyperparathyroidism.

Subgroup 2. Mean plasma calcium and phosphate levels remained unchanged during the period for the five children not treated with $1,25(\text{OH})_2\text{D}_3$ (10.2 ± 0.5 and 4.7 ± 0.9 mg/dl, respectively) and also the oral aluminum hydroxide dosage was not changed. There was a slight decrease in the incidence of hypercalcemic episodes compared with period I (from 9 to 6%) and the plasma calcium $\times$ phosphate product exceeded 60 in only 3% of the determinations. Despite the adequate plasma calcium and phosphate control mean plasma alkaline phosphatase and both COOH and NH_2 iPTH increased in all the children (Fig. 2); BMC did not change (Fig. 1).

One child of this group (patient 4) developed intense pruritus and hypertension after discontinuing $1,25(\text{OH})_2\text{D}_3$ supplementation. His plasma calcium and phosphate remained between 9.5 and 11.0 mg/dl during the period but plasma alkaline phosphatase increased from 44 to 160 U/liter (reference values in our control group 20–48 U/liter). Both NH_2 and COOH terminal fragments of iPTH greatly increased: iPTH-COOH from 1,300 to 10,980 pg/ml (reference values under 500 pg/ml) and iPTH-

NH_2 from 90 to 953 pg/ml (reference values under 200 pg/ml). His hypertension was moderate (mean value of all the determinations obtained prior to each HF session 150/100) and an antihypertensive drug (hydralazine 3 mg/kg/day) was prescribed. One month after the reintroduction of $1,25(\text{OH})_2\text{D}_3$ therapy at a dose of $0.50 \mu\text{g/day}$ plasma calcium increased from 10.0 to 10.5 mg/dl, plasma phosphate decreased from 5.4 to 4.9 mg/dl, and plasma alkaline phosphatase fell from 160 to 90 U/l. There was a decrease in both iPTH-COOH (3600 pg/ml) and iPTH- NH_2 (300 pg/ml). Blood pressure returned to normal (130/84) and the antihypertensive drug was discontinued.

Acute study. Total calcium balance in the 12 studies was positive with a total calcium gain per HF session of 139.8 ± 37.0 mg (Table 3). The total phosphate removed was estimated to be 573.8 ± 70.0 mg per HF session. Plasma total calcium significantly increased at the end of the session compared with the beginning while there was a significant decrease in plasma phosphate (from 4.6 ± 0.9 to 1.9 ± 0.4 mg/dl; $p < 0.001$) (Table 3). No significant change was observed in plasma total protein concentration and hematocrit.

DISCUSSION

The present results in children with ESRD treated with $1,25(\text{OH})_2\text{D}_3$ and HF showed that the biohumoral parameters of mineral metabolism and BMC were well controlled. Although we cannot compare the effect of $1,25(\text{OH})_2\text{D}_3$ in HD and HF, it appears that the administration of this drug to children on chronic HF may control renal osteodystrophy as well as during HD. Partial suppression of the hypocalcemic and hyperphosphatemic stimuli (21–23) and probably the direct action of $1,25(\text{OH})_2\text{D}_3$ on the parathyroid gland (23, 24) account for the control of iPTH during the study. During the first 6 months of HF and $1,25(\text{OH})_2\text{D}_3$ the significant decrease in mean plasma phosphate concentration associated with a decreased oral aluminum hydroxide requirement cannot be explained by the direct effect of $1,25(\text{OH})_2\text{D}_3$ therapy. In fact it is well known that $1,25(\text{OH})_2\text{D}_3$ increases intestinal absorption of phosphorus and

Fig. 1. Variations in BMC at the beginning of the study (values considered to be 100%), at 6 and at 12 months. a, subgroup 1 [HF and $1,25(\text{OH})_2\text{D}_3$]; b, subgroup 2 (HF alone).

Table 3. Plasma calcium, phosphate and total protein levels and hematocrit in six uremic children at the beginning (A) and end (B) of 12 HF sessions (values expressed as means $\pm$ SD)

	A	B	P
Plasma calcium (mg/dl)	9.9 ± 0.6	10.8 ± 0.6	<0.02
Plasma phosphate (mg/dl)	4.6 ± 0.9	1.9 ± 0.4	<0.001
Plasma total protein (g/l)	6.7 ± 0.8	6.7 ± 0.5	NS
Hematocrit (%)	18.1 ± 2.3	18.5 ± 2.3	NS

Fig. 2. Variations in alkaline phosphatase and iPTH-COOH and NH_2 in the second study period. The values at the beginning of this period are considered to be 100%. a, subgroup 1 [HF and $1,25(\text{OH})_2\text{D}_3$]; b, subgroup 2 (HF alone).

phosphatemia (24). Probably the decrease in plasma phosphate level was due both to the increase in plasma calcium levels and the high rate of phosphate removal obtained with our HF prescription (with a mean of 574 mg of phosphate removed during an HF run).

Also we studied the effect of HF on mineral balance without $1,25(\text{OH})_2\text{D}_3$ therapy. Results during 12 acute studies indicated that we obtained a positive calcium balance with a mean calcium gain of 140 mg during an HF session. This is in accordance with findings reported in studies of adults using HF infusion bags with a high calcium content ($\text{Ca}^{++} > 8.0 \text{ mg/dl}$) (26, 27). This result associated with the high phosphate removal obtained with our HF prescription suggests an improvement of secondary hyperparathyroidism in these patients. However, during our long-term study in all five of the subgroup 2 children, hyperparathyroidism worsened and BMC decreased despite good calcium-phosphate control. In contrast, hyperparathyroidism in the three patients who continued $1,25(\text{OH})_2\text{D}_3$ treatment improved further during the second study period. The key role of $1,25(\text{OH})_2\text{D}_3$ supplementation in these children is supported by the worsening hyperparathyroidism observed in patient 4 during the second study period accompanied by the development of pruritus and hypertension when vitamin D supplementation was discontinued; these changes were promptly reversed by the reintroduction of $1,25(\text{OH})_2\text{D}_3$.

In conclusion, our study indicates that renal osteodystrophy is not controlled by HF alone. $1,25(\text{OH})_2\text{D}_3$ supplementation is necessary to normalize the biohumoral parameters of calcium and phosphate metabolism and improve BMC.

REFERENCES

- Pierides AM, Ellis HA, Dellagrammatikas H, Scott JE, Norman AW 1977 $1,25$ -Dihydroxycholecalciferol in renal osteodystrophy. *Arch Dis Child* 52:464-472
- Bulla M, Delleng G, Offermann G, Ziegler R, Benz G, Luhmann H, Sanchez De Reutter M, Severin M 1979 Renal bone disorder in children: therapy with D_3 or $1,25$ -dihydroxycholecalciferol. *Proc Eur Dial Transplant Assoc* 16:644-648
- Chesney RW, Hamstra A, Jax JK, Mazess RB, DeLuca HF 1980 Influence of long-term oral $1,25$ -dihydroxyvitamin D in childhood renal osteodystrophy. *Contrib Nephrol* 18:55-71
- Chan JCM, Kodroff MB, Landwehr DG 1981 Effects of $1,25$ -dihydroxyvitamin- D_3 on renal function, mineral balance, and growth in children with severe chronic renal failure. *Pediatrics* 68:559-571
- Robitaille P, Marie PJ, Delvin EE, Lortie L, Glorieux FH 1984 Renal osteodystrophy in children treated with $1,25$ -dihydroxycholecalciferol ($1,25(\text{OH})_2\text{D}_3$). *Acta Paediatr Scand* 73:315-324
- Chan JCM, Young RB, Alon U, Manunes P 1983 Hypercalcemia in children with disorders of calcium and phosphate metabolism during long-term treatment with $1,25$ -dihydroxyvitamin- D_3 . *Pediatrics* 72:225-233
- Paunier L, Salusky IB, Slatopolsky E, Kangaroo H, Kopple JD, Horst RL, Coburn JW, Fine RN 1984 Renal osteodystrophy in children undergoing continuous ambulatory peritoneal dialysis. *Pediatr Res* 18:742-747
- Henderson LW, Colton CK, Ford CA 1975 Kinetics of hemodiafiltration II. Clinical characterization of a new blood cleansing modality. *J Lab Clin Med* 85:372-391
- Muller-Wiefel DE, Rauh W, Wirgen AM 1982 Hemofiltration in children. *Contrib Nephrol* 32:128-139
- Colton CK, Henderson LW, Ford CA, Lysaght MJ 1975 Kinetics of hemodiafiltration I. In vitro transport characteristics of a hollow-fiber blood ultrafilter. *J Lab Clin Med* 85:355-371
- Hampi H, Paepreer H, Unger V, Fischer C, Resa I, Kessel M 1980 Hemodynamic changes during hemodialysis, sequential ultrafiltration and hemofiltration. *Kidney Int* 18:S-83-88
- Edefonti A, Saccaggi A, Galato R, Ghio L, Giani M, Picca M, Capitanio L, Bettinelli A 1984 Haemofiltration in children. In: Brodehl J, Ehrich JK (eds) *Paediatric Nephrology*. Springer-Verlag, New York, pp 106-109
- Quellhorst E, Schenunemann B, Borghardt J 1978 Clinical and technical aspects of hemofiltration. *Dial Transplant* 7:4-12
- Schneider H, Streicher E, Schmidt-Gayk H 1979 Does long-term hemofiltration provoke secondary hyperparathyroidism? *Dial Transplant* 8:1004-1012
- De Fremont JF, Moriniere P, Guerin J, Gheerbrandt JD, Coevoet B, Fournier A 1981 Hemofiltration does not worsen or improve hyperparathyroidism. XVIII Eur Dial Transplant Assoc 25(abstr)
- Sargent J, Gotch F, Borah M, Pierc YL, Spinuzzi N, Schoenfeld P, Humphreys M 1978 Urea kinetics: a guide to nutritional management of renal failure. *Am J Clin Nutr* 31:1696-1702
- Cecchetti M, Albertin A, Zoppi G 1982 Radioimmunological methods in diagnosis of nutritional disturbances in infancy. In: Zoppi G (ed) *Metabolic-endocrinological Responses to Food Intake in Infancy*. Karger, Basel, pp 6-20
- Asknes L 1980 Quantitation of the main metabolites of vitamin D in a single serum sample. I. Extraction, separation and purification of metabolites. *Clin Chim Acta* 104:133-146
- Asknes L 1980 Quantitation of the main metabolites of vitamin D in a single serum sample. II. Determination by UV-absorption and competitive protein binding assays. *Clin Chim Acta* 104:147-161
- Bianchi ML, Bettinelli A, Aimini E, Edefonti A, Soldati L, Quinzanini M, Cecchetti M 1983 $1,25$ -Dihydroxycholecalciferol in the treatment of renal osteodystrophy in children. In: Caniggia A (ed) *Proceeding of the 4th International Congress on Calcitropic Hormones*. Florence, pp 77-79
- Goldstein DA, Malluche HH, Massry SG 1979 Management of renal osteodystrophy with $1,25(\text{OH})_2\text{D}_3$: I. Effects on clinical, radiographic and biochemical parameters. *Miner Electrolyte Metab* 2:35-43
- Brickman AS, Coburn JW, Sherrard DJ 1980 Clinical effects of $1,25$ -dihydroxyvitamin- D_3 in uremic patients with overt osteodystrophy. *Contrib Nephrol* 18:29-45
- Slatopolsky E, Weerts C, Thielan J, Horst R, Harter H, Martin K 1984 Marked suppression of secondary hyperparathyroidism by intravenous administration of $1,25$ -dihydroxy-cholecalciferol in uremic patients. *J Clin Invest* 74:2136-2145
- Golden P, Mazey R, Greenwalt A 1979 Vitamin D: a direct effect on parathyroid gland? *Miner Electrolyte Metab* 2:1-20
- Brickman AS, Hartenbower DL, Norman AW, Coburn JW 1977 Actions of 1 -hydroxyvitamin D_3 and $1,25$ -dihydroxyvitamin D_3 on mineral metabolism in man. Effects on net absorption of phosphorus. *Am J Clin Nutr* 30:1064
- Bosch JP, Von Albertini B, Geronemus R 1981 Comparison of hemofiltration and ultrafiltration plus hemodialysis to conventional hemodialysis. Final Report No 1-AM-7-2228, Artificial Kidney Chronic Uremia Program, NIH, Bethesda
- Quellhorst E, Scheunemann B 1978 Metabolic and hemodynamic aspects of hemofiltration. *Dial Transplant* 7:369-382