Clearance of Osteocalcin by Peritoneal Dialysis in Children with End-Stage Renal Disease

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ABSTRACT. Osteocalcin is a bone-specific protein whose concentration in blood is a direct reflection of bone turnover. In chronic renal failure, circulating osteocalcin is elevated. This elevation is due to decreased renal clearance and, in some patients, increased bone turnover secondary to renal osteodystrophy. In children receiving continuous ambulatory peritoneal dialysis, mean serum osteocalcin concentrations are substantially lower than in similar patients on hemodialysis (1). This difference may be due to clearance of the protein by the peritoneal membrane. To test this possibility we examined osteocalcin in 16 infants and adolescents undergoing continuous ambulatory peritoneal dialysis with two commercially available glucose-based dialysis solutions (2.5 and 4.25% Dianeal). Mass transfer of osteocalcin over 5-h dialysis exchange periods was -18.9 ± 2.8 and $-28.4 \pm 7.8 \mu\text{g}$ for the low and high glucose solutions, respectively. Serum levels fell over the course of single exchange periods in concert with increasing dialysate concentrations. There were significant correlations between initial blood concentrations of osteocalcin and the total amount of osteocalcin transferred ($r = 0.609$ and 0.642 for the high and low glucose solutions, respectively, $p < 0.05$). There were also strong correlations between the mass transfers of osteocalcin and those of creatinine ($p < 0.05$) and total protein ($p < 0.01$) with the 4.25% glucose exchange. The relationships were weaker with the 2.5% glucose exchange. Fractionation of serum revealed a single immunoreactive peak eluting coincident with intact osteocalcin, but two or three immunoreactive peaks were identified in matching dialysate samples, suggesting that both intact osteocalcin and circulating fragments are transferred by the peritoneal membrane. The efficient clearance of the protein or its metabolic fragments may be a significant factor in the natural history of renal osteodystrophy seen in these patients. (*Pediatr Res* 21: 296-300, 1987)

HPLC, high-pressure liquid chromatography
PTH, parathyroid hormone
GFR, glomerular filtration rate

Osteocalcin is a small protein (5800 daltons) synthesized in bone. It is characterized by the presence of three residues of Gla, an amino acid formed posttranslationally by vitamin K-dependent carboxylation of specific glutamic acid residues in the protein (2). These Gla residues allow osteocalcin to bind specifically and avidly to hydroxyapatite, the principal mineral phase of bone (3). During bone formation, a portion of the newly synthesized protein is secreted into the circulation, where it can be measured by radioimmunoassay. The circulating fraction of osteocalcin is normally filtered by the kidney where it is catabolized to its constituent amino acids (4, 5). Increases in serum osteocalcin concentration have been observed in infants and children with disorders characterized by increased bone turnover (1, 6). Correlation with quantitative bone histology shows that serum osteocalcin is a measure of the rate of bone formation (7), but recent evidence suggests that serum concentrations of osteocalcin are dependent on renal clearance as well (8, 9).

Growth retardation and defective bone remodeling are common and serious complications of chronic renal failure in children. While hemodialysis has been the mainstay of supportive therapy, CAPD is now an important alternative therapy. Some have suggested that it offers a more constant control of biochemical parameters than does hemodialysis (10) and that it may be effective in the prevention of radiologically demonstrable bone disease in children (11). However, studies in both children and adults have shown that the benefits of CAPD are not uniform and in some cases, bone lesions may progress (11, 12).

We have previously shown that in children with renal failure, circulating osteocalcin is substantially increased. Children receiving CAPD, however, had mean serum osteocalcin concentrations that were at least 50% lower than levels in similar children receiving hemodialysis, although still greater than those of age-matched controls (1). Lower concentrations of serum osteocalcin with CAPD may be explained by the high concentration of the protein in peritoneal dialysate compared to nondetectable levels in hemodialysate. The aims of the present study were therefore: 1) to determine the clearance of osteocalcin by CAPD and compare it to other parameters of calcium metabolism in children, 2) to evaluate the effect of CAPD on osteocalcin metabolism, and 3) to provide additional information on the removal of low molecular weight proteins from the circulation through the peritoneal membrane.

Abbreviations

Gla, γ -carboxyglutamic acid
CAPD, continuous ambulatory peritoneal dialysis

Received January 21, 1986; accepted October 29, 1986.

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Supported in part by NIH Grant AM35407. D.E.C.C. is a recipient of a scholarship from the Canadian Life and Health Insurance Association.

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Table 1. *Clinical variables and baseline values*

Age/sex (yr)	Diagnosis	Renal osteodystrophy*	Growth velocity†	Treatment		Immunoreactive PTH (pmol/ liter)	Calcium (mg/dl)	Phosphate (mg/dl)	Alkaline phosphatase (IU/ liter)	Creatinine (mg/dl)	Osteocalcin (μg/liter)
				1,25(OH) ₂ D ₃ (μg/day)	Phosphate binder						
INFANTS											
0.5/M	Hemolytic-uremic syn- drome	+	Normal	Vitamin D	Amphogel	9	6.4	8.0	415	3.5	23
0.7/M	Renal artery thrombosis	++	Normal	1.0	Amphogel	ND	8.5	6.8	74	3.4	27
1.1/M	Dysplastic kidneys	++	Normal	1.0	Amphogel	112	8.2	4.6	486	3.4	55
1.3/M	Hypoplastic/dysplastic kidneys	++	Normal	0.25	Amphogel	124	9.3	6.3	143	5.3	77
1.9/M	Prune-belly syndrome	++	Normal	0.25	Amphogel	56	8.0	2.8	820	5.5	23
Normal val- ues						<3–25	9–11	3.3–6.0	F:185–555 M:175–600	<0.5	10–20
Children and adolescents											
9.3/F	Glomerular nephritis- undiff.	0	Poor	0.25	Basogel	33	7.6	6.0	111	5.5	25
9.7/M	MPGN-type II	0	Fair	0.25	Basogel	63	9.6	6.0	94	13.8	50
10.3/M	Focal glomerulosclerosis	+	Poor	0.25	Amphogel	39	7.8	6.0	130	6.0	94
10.3/M	Dysplastic/hypoplastic kidneys	0	ND‡	0.25	Basogel	ND	9.3	4.9	264	5.9	129
10.3/F	Congenital nephrotic syndrome	0	ND‡	0.25	None	27	9.6	5.3	322	9.3	56
12.4/F	Nephronophthisis	0	Poor	0.50	Basogel	16	9.0	5.6	239	7.6	71
13.7/M	Henoch-Schonlein ne- phritis	0	Fair	0.25	Amphogel	29	9.2	7.5	112	9.9	68
15.5/M	MPGN-type I	0	Normal	0.25	Amphogel	102	8.1	5.5	145	10.2	169
15.8/M	MPGN-type II	0	Normal	0.50	Amphogel	26	8.6	2.8	389	7.7	155
18.0/M	Obstructive uropathy	0	Normal	0.25	Basogel	2	8.0	6.5	174	11.4	35
19.2/F	Medullary cystic disease	0	Normal	0.25	Amphogel	ND	9.4	4.6	29	13.3	26
Normal val- ues						<3–25	9–11	3.2–5.4	F(9–13)160–500 (14–18)45–140 M(9–14)200–630 (15–18)80–210	<1.2	F:10–40 M:20–80

* Renal osteodystrophy was graded as follows: 0: no radiological evidence, +: minimal changes, ++: marked undermineralization and rachitic changes.

† Growth velocities were defined as normal, fair, or poor when they were –0.19 to +0.19 SD, –0.2 to –0.49 SD, or more than –0.5 SD respectively, of the growth velocities of normal children of the same age and sex.

‡ Not determined.

METHODS

Patients. The study protocol has been described previously (13). Informed parental consent was obtained for all subjects. The research protocol was approved by the Human Subjects Review Committee of the Hospital for Sick Children, Toronto, Ontario, where the patients were enrolled in an established CAPD program. The studies took place during the latter part of the CAPD training program, by which time all patients were tolerating appropriate dialysate volumes. All patients had been followed for at least 18 months and were anuric or oliguric, and none had undergone parathyroidectomy. All were free of other medical complications. Patients were fasted from the preceding midnight until the end of each study and were dialyzed overnight with 2.5% glucose dialysate. The studies took place during the 5-h morning dialysis exchange, starting at 0700 h. Simultaneous blood and peritoneal fluid samples were taken immediately before, and at 1 and 5 h following the infusion of 2.5 and 4.25% glucose-based dialysis solutions. (Dianeal, Baxter-Travenol Canada Ltd., Malton, Ontario, Canada).

To ensure sterility, dialysate samples were obtained by allowing approximately 30 ml of peritoneal fluid to drain from the patient into the bag. A further 10 ml of fluid were then drained from the patient into a receptacle (Buritrol, Baxter-Travenol) which was attached to the injection port of the dialysate bag. The Buritrol connection was then closed, the aliquot removed from the Buritrol, and the initial 30 ml fluid volume in the bag returned to the patient. At the end of the 5-h exchange, the peritoneal cavity was completely drained of dialysate, the volume measured, and aliquot samples taken.

Fasting and initial biochemical and clinical variables of the patients are given in Table 1.

Laboratory procedures. Plasma and peritoneal calcium, creatinine, alkaline phosphatase, and total protein were measured by standard laboratory techniques. PTH measurements were conducted in the laboratory of Dr. T. M. Murray, Division of Endocrinology and Metabolism, St. Michael's Hospital, Toronto, Canada (14). Osteocalcin was measured by radioimmunoassay described in detail elsewhere (15).

Matched samples of serum and dialysate from three separate individuals were each subjected to Sephadex G75 gel filtration. Peak material containing immunoreactive osteocalcin was pooled and further characterized by HPLC. Effluent from HPLC was collected in 0.1-ml fractions and each fraction assayed for osteocalcin. The details of this method and results of such analysis of serum from normal adults and those with renal failure are described elsewhere (16).

Clearances were calculated with the general formula:

Clearance (ml/min)

$$= \frac{\text{dialysate concentration}}{\text{serum concentration}} \times \frac{\text{dialysate volume}}{\text{time (min)}}$$

Statistical analysis. Data are expressed as means $\pm$ SE. Correlation coefficients were calculated by Spearman's rank correlation. The mean differences between groups were tested using Student's two-tailed *t* test.

RESULTS

The clinical conditions, growth characteristics, treatment, and baseline biochemical values are given in Table 1. Patients were classified into two groups—an infant group (ages 6 to 20 months) with marked renal osteodystrophy and high vitamin D requirements, and an older group (ages 9.3 to 18 yr spanning adolescence) with mild renal osteodystrophy and lower vitamin D supplementation. Serum osteocalcin was significantly lower in infants than in children and adolescents (41 ± 9 versus 80 ± 15 $\mu\text{g/liter}$, $p < 0.01$), as was the serum creatinine (4.2 ± 0.4 versus 9.2 ± 0.8 mg/dl, $p < 0.01$). There was, however, no correlation between the two ($r = 0.092$). In all of the study subjects serum

osteocalcin was elevated for age; mean values for both groups were approximately twice the normal mean values. Immunoreactive PTH was elevated in both groups. Mean alkaline phosphatase activity was elevated in both groups, but did not correlate significantly with osteocalcin. Furthermore there were no significant correlations between osteocalcin and immunoreactive PTH, calcium, phosphate, or creatinine.

The effect of dialysis on individual serum and dialysate con-

Fig. 1. Changes in individual serum and dialysate osteocalcin concentrations during dialysis with either 2.5 or 4.25% glucose dialysates.

Fig. 2. Upper panel, osteocalcin concentration in dialysate (mean $\pm$ SE) relative to matching serum values. Lower panel, serum osteocalcin concentrations (mean $\pm$ SE) relative to predialysis values.

centrations of osteocalcin for both study solutions is shown in Figure 1. Serum osteocalcin decreased at 1 hr and rose again by 5 h, but remained below predialysis concentrations. Measurable amounts of osteocalcin were found in the dialysate at 1 h and levels were further increased at 5 h. Osteocalcin losses were considerably higher in the infant group ($1.5 \pm 0.5 \mu\text{g/kg}$) than in children and adolescents ($0.6 \pm 0.3 \mu\text{g/kg}$).

The relative changes in serum osteocalcin concentrations during CAPD with either low or high glucose solutions were similar (Fig. 2). However, the clearance of osteocalcin was greater in the high glucose than in the low glucose group (Table 2). Similarly, clearances of creatinine, calcium, and total protein were increased by dialysis with high glucose (Table 2). Osteocalcin clearance in the high glucose cycle was twice that of the low glucose cycle. Because there was a greater volume removed in the high glucose cycle (mean ± 1.38 liter) than in the low glucose cycle (mean = 0.96 liter), there was also a significant difference in the total mass transfer of osteocalcin (-18.9 ± 2.8 versus $-28.4 \pm 7.8 \mu\text{g}$ for the low and high glucose solutions, respectively, $p < 0.05$).

There were significant correlations between initial serum concentrations of osteocalcin and the total amount of osteocalcin transferred per exchange with both solutions (Table 3). However, no correlations were found between initial serum osteocalcin concentrations and the other serum baseline biochemical variables or the dialysate concentrations of calcium, creatinine, or total protein. There was also no correlation between dialysate osteocalcin and dialysate total calcium. There were significant correlations between the mass transfer of osteocalcin and that for total protein or creatinine. These correlations were always stronger in the high glucose study than in the low glucose study. Serum creatinine correlated with dialysate osteocalcin only in the 4.25% glucose cycle.

Because circulating osteocalcin in renal failure includes both intact protein and fragments (16), serum and dialysate was fractionated by gel chromatography and HPLC. One immuno-

Fig. 3. Matched sera (2 ml) and dialysate (50 ml) from three patients were each chromatographed by G75 gel filtration (column dimensions: 2.0×80 cm, eluting buffer: $0.05 \text{ M NH}_4\text{HCO}_3$). A single peak of immunoreactive osteocalcin was detected (data not shown). The peak material was pooled and subjected to HPLC, (shown here). In all three serum samples, only one peak of osteocalcin immunoreactivity (C), eluting coincident with authentic osteocalcin, was observed. In the dialysate samples, either two or three peaks were found. Table insert lists the peaks of cross-reactivity found in each sample.

reactive peak coeluting with intact osteocalcin was detected in all sera but two or three peaks were found in the dialysate (Fig. 3).

DISCUSSION

Circulating osteocalcin is catabolized by the kidney to its constituent amino acids and no detectable intact osteocalcin can be found in the urine (4). In progressive renal failure, there is an inverse relationship between serum osteocalcin and GFR (8). When renal function declines to less than $20 \text{ ml/min per } 1.73 \text{ m}^2$, serum concentrations increase and can be 20–50 times higher than normal. The interpretation of osteocalcin levels in those patients with renal osteodystrophy, therefore, is a product of at least two independent determining factors—decreased renal function and increased bone turnover (16–18). In the present study, we have measured the clearance of osteocalcin in 16 infants and children on CAPD. In these patients, osteocalcin did not correlate significantly with peritoneal clearance of creatinine, probably because the changes in the rate of skeletal turnover due to bone disease or growth were more important factors in the circulating concentration.

Serum osteocalcin concentrations were lower in infants than in older children and adolescents which may be explained in part by the normal variation of osteocalcin with age and linear growth rate (1). In children under the age of 10, normal osteocalcin values range from 10 to $20 \mu\text{g/liter}$, but serum osteocalcin is increased during pubertal growth spurts in boys and girls. In adolescent boys, mean concentrations may reach $50 \mu\text{g/liter}$, while in girls they are $25 \mu\text{g/liter}$ (1). The radiological evidence in this study confirms the suggestion that children with early onset renal failure have a higher incidence of bone disease (Table 1) (11). However, the increases in serum osteocalcin concentrations relative to age-related normal values were similar in both age groups.

We found no significant correlations between serum osteocalcin and any of the other baseline biochemical values. These included calcium, creatinine, alkaline phosphatase, or PTH. This

Table 2. Comparison of solute clearance with 2.5 and 4.25% glucose

	2.5% glucose	4.25% glucose
Dialysate volume (ml)	963 ± 124	1382 ± 130
(ml/kg)	32.1 ± 4.1	46.0 ± 4.3
Creatinine clearance (ml/min)	2.43 ± 0.25	3.61 ± 0.12
Calcium clearance (ml/min)	-0.376 ± 0.012	-0.687 ± 0.029
Total protein clearance (ml/min)	0.084 ± 0.017	0.151 ± 0.019
Osteocalcin clearance (ml/min)	0.98 ± 0.11	1.94 ± 0.24

Table 3. Correlations between osteocalcin and other biochemical parameters with 2.5 and 4.25% glucose

	Serum osteocalcin		Dialysate osteocalcin	
	4.25%	2.5%	4.25%	2.5%
Dialysate				
Osteocalcin	0.609*	0.642*		
Calcium	0.423	0.161	0.274	0.168
Creatinine	0.022	0.054	0.648*	-0.482
Total protein	-0.050	0.486	0.716†	0.466
Serum				
Calcium	-0.099	0.307	0.230	0.092
Creatinine	0.060	0.253	0.566*	0.192
Alkaline phosphatase	0.209	-0.083	0.199	0.063
PTH	-0.069	-0.126	-0.042	0.130

* $p < 0.05$.

† $p < 0.01$.

is in contrast to the findings of Epstein *et al.* (17) who reported a significant correlation between osteocalcin and alkaline phosphatase in eight adults on CAPD. This may be due to different specificities of the respective antibodies or to differences in the adult *versus* juvenile populations.

Others have found a correlation between serum osteocalcin and serum creatinine in adults with predialysis renal failure or in those undergoing hemodialysis (8, 17–19). As in our study of children on CAPD, Epstein *et al.* (17) found no correlation between serum osteocalcin and creatinine in adults on CAPD. We observed that creatinine and osteocalcin clearances were correlated only in the high glucose dialysate but not in the low glucose dialysate. Since our patients had been maintained on 2.5% glucose, it is probable that the differing clearance rates for the two solutes accounted for the lack of correlation between the serum values in the baseline state.

Our previous studies show that serum osteocalcin levels in juvenile CAPD patients are lower than those in hemodialysis patients (1). Significant amounts of osteocalcin were detected in the peritoneal dialysate but none could be found in the hemodialysate. In the present study, we have shown that CAPD removes a significant amount of osteocalcin from the circulation and that osteocalcin is removed in proportion to its concentration in serum. Serum levels fall in concert with rising dialysate concentrations at 1 h, but rise again at 5 h. This subsequent rise is presumably due to the continued release of osteocalcin into the circulation coupled with the declining efficiency of the dialysis. As expected, the increased infusate osmolality and effluent dialysate volume with the 4.25% glucose solution resulted in greater mass transfer of osteocalcin than that observed with the 2.5% solution.

During osteoclastic bone resorption, proteolytic fragments of osteocalcin, some of which may carry the antigenic determinant, may be released into the circulation. Our antibody can detect C-terminal fragments of osteocalcin when they are at least 15 residues in length and in high concentrations. With renal failure, detectable fragments which are normally cleared will accumulate. In normal adults and children a single immunoreactive peak of osteocalcin is obtained from sera subjected to sequential gel filtration and HPLC. On the other hand, several immunoreactive peaks are observed in the sera of adults on hemodialysis (16). It is therefore of interest that we were able to detect only intact osteocalcin in the sera of CAPD patients, while two or three peaks were found in the dialysates. Since intact osteocalcin is cleared by the peritoneal membrane, any circulating fragments should also be transferred. Presumably the major fragments identified in the dialysate are rapidly cleared from the serum so that their concentration in serum is below the detectable limit of our assay. Peritoneal dialysate therefore provides a convenient source of circulating protein metabolites which are otherwise rapidly degraded and excreted by the kidney.

Loss of proteins into the peritoneal dialysate is dependent on molecular weight, shape, and net charge (20, 21). Osteocalcin has a molecular mass of 5800 daltons but is a very highly charged protein (isoelectric point = 3.5). Its diffusion characteristics may be more typical of a larger molecule because of this. Nevertheless, its clearance relative to creatinine is high, reaching 54% of that for creatinine ($M_r = 113$). It is likely that the high clearance rate is due to the transfer of substantial amounts of smaller immunoreactive osteocalcin fragments. Immunoreactive PTH has also been identified in the serum and dialysate of CAPD patients (22). As with osteocalcin, its peritoneal clearance is high relative to other proteins. This observation may also apply to other proteins that undergo proteolytic degradation.

We have demonstrated that CAPD removes substantial quantities of osteocalcin and its metabolic fragments. Although the physiologic role for osteocalcin is not defined, it has been demonstrated that both the intact protein and its C-terminal penta-

peptide are chemotactic for monocytes (23, 24). This property may be central to recruitment, differentiation, and regulation of bone resorbing cells. Furthermore, osteocalcin is considered to be an inhibitor of mineralization (25). Thus, the efficient clearance of the protein or its metabolic fragments may be a significant factor in the natural history of renal osteodystrophy seen in these patients.

Acknowledgment. The authors thank Jenifer Aronoff for technical assistance.

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